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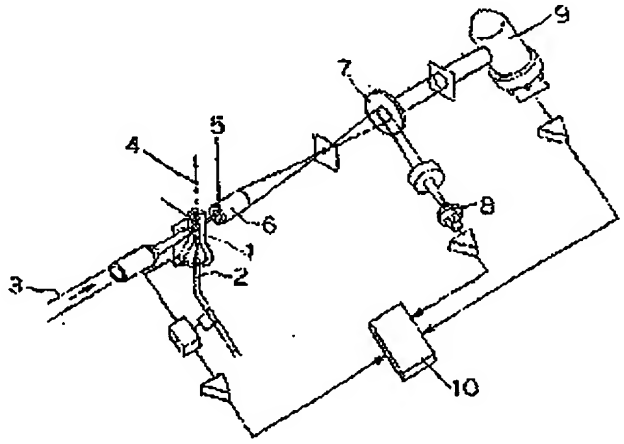
(54)【発明の名称】　微生物測定方法及び装置

(57)【要約】
【課題】　微生物の測定における夾雑物の影響を削減し、測定に要する時間を削減する。
【構成】　培養前と培養後とで微生物の測定を行い、両者の差を取る。これにより、検体中の夾雑物の影響による誤測定を防止する。フローサイトメータにより微生物の測定を行うため、培養時間が短くても微生物を測定できる。しかも、夾雑物を測定しないため、測定が正確である。さらに、フローサイトメータにより検出される前方散乱光の発光時間に対する発光強度の変化を測定することで、微生物の増殖形態が分かる。従って、粒度分布の培養前後における差から、微生物を、桿菌、ブドウ球菌、連鎖桿菌、連鎖球菌及び酵母様真菌の５つに大別可能である。



1

【特許請求の範囲】

【請求項１】測定対象の検体を培養液に加えた培養試料中の微生物の測定方法であって、

所定時間培養処理した後の前記培養試料を前記フローサイトメータを用いて測定し、検出される散乱光の光学的情報から前記培養試料の第１粒度分布を得る第１測定工程と、

前記培養処理前の培養試料を前記フローサイトメータを用いて測定し、検出される散乱光の光学的情報から前記培養試料の第２粒度分布を得る第２測定工程と、

前記第１粒度分布と第２粒度分布との差分から、前記培養試料中で培養された微生物の粒度分布を得る解析工程と、

前記得られた微生物の粒度分布を出力する出力工程と、を含むことを特徴とする微生物測定方法。

【請求項２】前記フローサイトメータを用いて検出される散乱光信号の発光時間と強度とを測定し、前記第１及び第２粒度分布を得ることを特徴とする、請求項１記載の微生物測定方法。

【請求項３】前記フローサイトメータを用いて検出される散乱光信号の発光時間と強度とを測定することにより前記培養された微生物の粒度分布を得、前記粒度分布に基づいて前記微生物の増殖形態を推定し、所定の分類のいずれに前記微生物が属するかを判定することを特徴とする、請求項１記載の微生物測定方法。

【請求項４】前記フローサイトメータを用いて検出される散乱光信号の発光時間と強度とを測定することにより前記培養された微生物の粒度分布を得、前記微生物の増殖形態がブドウ球菌、連鎖球菌、連鎖桿菌または桿菌のいずれに属するかを前記粒度分布に基づいて判定することを特徴とする、請求項１記載の微生物測定方法。

【請求項５】測定対象検体が尿であることを特徴とする、請求項１記載の微生物測定方法。

【請求項６】微生物として、細菌及び／または酵母様真菌を測定することを特徴とする請求項１記載の微生物測定方法。

【請求項７】測定対象検体を培養液に加えた培養試料中の微生物の粒度を測定するフローサイトメータとともに用いられ、

前記フローサイトメータにより検出される散乱光の光学的情報から、所定時間培養処理を行った後の培養試料の第１粒度分布を得る第１測定手段と、

前記フローサイトメータにより検出される散乱光の光学的情報から、前記培養処理前の培養試料の第２粒度分布を得る第２測定手段と、

前記第１粒度分布と第２粒度分布との差分から、前記培養試料中で培養された微生物の粒度分布を得る解析手段と、

前記得られた微生物の粒度分布を出力する出力手段と、を備えることを特徴とする微生物測定装置。

2

【請求項８】測定対象検体を培養液に加えた培養試料中の微生物の粒度を測定するフローサイトメータとともに用いられ、前記粒度の測定結果を解析する解析プログラムを記録したコンピュータ読み取り可能な記録媒体であって、

Ａ；前記フローサイトメータにより検出される散乱光の光学的情報から、所定時間培養処理を行った後の培養試料の第１粒度分布を得る段階と、

Ｂ；前記フローサイトメータにより検出される散乱光の光学的情報から、前記培養処理前の培養試料の第２粒度分布を得る段階と、

Ｃ；前記第１粒度分布と第２粒度分布との差分に基づいて、前記培養試料中で培養された微生物の粒度分布を得る段階と、

Ｄ；前記得られた微生物の粒度分布を出力する段階と、を実行するための解析プログラムを記録した、コンピュータ読み取り可能な記録媒体。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、細菌その他培養可能な微生物を培養して菌数を測定する方法に関する。なかでも、微生物以外の夾雑物が含まれる試料においても、短時間の培養で高感度に菌数を測定する微生物測定方法に関する。

【０００２】

【従来の技術】従来、微生物を培養して微生物の数を測定する一般的な方法として、寒天平板塗抹法がある。この方法では、適度な栄養分を含む寒天平板培地上に一定量の検体を塗抹し、コロニーが肉眼又は顕微鏡で観察できる大きさになるまで培養した後、生じたコロニー数を計測する。しかし、この方法を用いると、肉眼又は顕微鏡で観察可能となる程度まで菌コロニーが成長するのを待たねばならない。そのため、菌の種類をある程度類推するために、通常１８～２４時間程度の培養期間を要している。菌種によっては、培養期間は２４時間以上、４８時間以上、あるいは１月など長期に渡るものもある。

【０００３】培養により増殖した菌の種類によっては、微生物を特定するための菌種の同定検査や薬剤の有効性を調べる薬剤感受性検査が必要になる場合がある。ところが、前記方法では、これらの必要性が判明するまでに時間がかかり、たくさんの試料を効率的に検査することが難しい。前述の寒天平板培地のかわりに液体培地を用いて試料を培養する方法がある。この方法は、適度な栄養分を含む液体培地に一定量の検体を混和して培養を行ない、肉眼や吸光度高度計、分光光度計などで濁度を測定する。しかし、光度計は、感度がいいとは言えないため、この方法においても比濁が変化する程度まで菌の増殖を待たねばならない。そのため、菌数の測定には、寒天平板塗抹法と同様に、何日間かの培養期間が必要であり、前述の問題がある。

【０００４】また、特開平５－８２９０１号公報には、培養中の検体に光を照射し、散乱光及び透過光の時間に対する変化から菌数を測定する方法が開示されている。この方法は、発育形態が異なる菌であっても正確に菌数を求めることができる。しかし、この方法は、菌の発育課程における散乱光データの変化や透過光データの変化を用いるため、検体の培養に要する時間は短縮されない。

【０００５】

【発明が解決しようとする課題】一方、微生物のような微小な大きさの粒子を高感度に測定する装置として、フローサイトメータ等の粒子測定装置が知られている。粒子測定装置は、試料中の粒子の数を１つ１つ計測するため、高感度な測定が可能である。従って、粒子測定装置を用いれば、長時間培養しなくとも試料中の細菌数を計測可能である。

【０００６】しかし、粒子測定装置は、微生物と同じくらいの大きさの粒子、例えば埃や析出物などの夾雑物を、微生物として検出してしまう。このため、粒子測定装置の検出結果には、夾雑物による誤差が含まれてしまう。例えば尿を検体として細菌を測定する際、その尿中の有形成分である赤血球、白血球、上皮細胞、円柱、結晶等やその崩壊物が細菌として検出されてしまう。

【０００７】そこで、夾雑物と微生物とを識別するために、微生物を染色し、微生物から発せられる蛍光を測定する方法が提案されている。しかし、微生物の種類によって染色度合いが異なる。また、目的とする微生物のみを染色する蛍光染料や処理条件を試料ごとに設定しなくてはならない。このため、測定に手間がかかり、大量の検体を分析するには不向きである。

【０００８】他に微生物数を迅速に検出する方法として、微生物の増殖に伴う培地のインピーダンスの変化、培養液のＰＨの変化、消費酸素量あるいは発生炭酸ガス量等を測定し、これらと微生物数の相関から微生物数を求める方法が最近研究されている。しかし、培養された微生物以外の原因によって前記測定値は変化することがある。また、微生物の検出限界、検出精度の点から満足できる方法とはいえないため、特定の条件下のみでしか使えない方法である。すなわち、前述のように、検体中の微生物を、短時間にかつ正確に判定可能な方法は、未だ提供されていない。

【０００９】本発明は、以上のような事情を考慮したもので、微生物の測定を短時間の培養で簡易に精度良く測定する方法を提供することを目的とする。

【００１０】

【課題を解決するための手段】本願第１発明は、上記の課題を解決するため、測定対象の検体を培養液に加えた培養試料中の微生物の測定方法であって、

Ａ；所定時間培養処理した後の前記培養試料を前記フローサイトメータを用いて測定し、検出される散乱光の光学的情報から前記培養試料の第１粒度分布を得る第１測定工程と、

Ｂ；前記培養処理前の培養試料を前記フローサイトメータを用いて測定し、検出される散乱光の光学的情報から前記培養試料の第２粒度分布を得る第２測定工程と、

Ｃ；前記第１粒度分布と第２粒度分布との差分から、前記培養試料中で培養された微生物の粒度分布を得る解析工程と、

Ｄ；前記得られた微生物の粒度分布を出力する出力工程と、

を含むことを特徴とする微生物測定方法を提供する。

【００１１】培養後の測定結果から培養前の測定結果を減算することにより、検体に含まれている夾雑物を微生物として測定することを防止することが出来る。夾雑物とは、例えば埃や検体中の有形成分である。本願第２発明は、前記第１発明において、前記フローサイトメータを用いて検出される散乱光信号の発光時間と強度とを測定し、前記第１及び第２粒度分布を得ることを特徴とする微生物測定方法を提供する。

【００１２】散乱光強度は、培養試料に含まれる粒体の大きさに関する情報を与える。また、散乱光発光時間は、培養試料に含まれる粒体の長さに関する情報を与える。従って、発光時間に対する強度の変化を測定することにより、鎖状に増殖したり、ブドウの房状に増殖しているなどの増殖形態を知ることが出来る。本願第３発明は、前記第１発明において、前記フローサイトメータを用いて検出される散乱光信号の発光時間と強度とを測定することにより、前記培養された微生物の粒度分布を得、前記粒度分布に基づいて前記微生物の増殖形態を推定し、所定の分類のいずれに前記微生物が属するかを判定することを特徴とする微生物測定方法を提供する。

【００１３】径が比較的大きく長さがさほど長くない菌が増殖している場合はブドウ球菌、径が比較的小さく長さが短い菌が増殖している場合は桿菌、径が比較的小さく長い菌が増殖している場合は連鎖桿菌、径が連鎖桿菌よりもさらに小さく長い菌が増殖している場合は連鎖球菌と推定できる。本願第４発明は、前記第１発明において、前記フローサイトメータを用いて検出される散乱光信号の発光時間と強度とを測定することにより前記培養された微生物の粒度分布を得、前記微生物の増殖形態がブドウ球菌、連鎖球菌、連鎖桿菌または桿菌のいずれに属するかを前記粒度分布に基づいて判定することを特徴とする微生物測定方法を提供する。

【００１４】前記第３発明と同様の作用効果を有する。本願第５発明は、前記第１発明において、測定対象検体が尿であることを特徴とする微生物測定方法を提供する。尿を検体とする場合、前記微生物の分類結果からグラム陰性／陽性の判断が可能である。

【００１５】本願第６発明は、前記第１発明において、微生物として、細菌及び／または酵母様真菌を測定する

ことを特徴とする微生物測定方法を提供する。本願第７発明は、測定対象検体を培養液に加えた培養試料中の微生物の粒度を測定するフローサイトメータとともに用いられ．第１測定手段と、第２測定手段と、解析手段と、出力手段とを備えることを特徴とする微生物測定装置を提供する。第１測定手段は．前記フローサイトメータにより検出される散乱光の光学的情報から、所定時間培養処理を行った後の培養試料の第１粒度分布を得る。第２測定手段は、前記フローサイトメータにより検出される散乱光の光学的情報から．前記培養処理前の培養試料の第２粒度分布を得る。解析手段は、前記第１粒度分布と第２粒度分布との差分から．前記培養試料中で培養された微生物の粒度分布を得る。出力手段は、前記得られた微生物の粒度分布を出力する。

【００１６】前記第１発明と同様の作用効果を有する。本願第８発明は．測定対象検体を培養液に加えた培養試料中の微生物の粒度を測定するフローサイトメータとともに用いられ．前記粒度の測定結果を解析する解析プログラムを記録したコンピュータ読み取り可能な記録媒体であって、下記Ａ～Ｄ段階を実行するための解析プログラムを記録した．コンピュータ読み取り可能な記録媒体を提供する。

Ａ；前記フローサイトメータにより検出される散乱光の光学的情報から．所定時間培養処理を行った後の培養試料の第１粒度分布を得る段階、

Ｂ；前記フローサイトメータにより検出される散乱光の光学的情報から．前記培養処理前の培養試料の第２粒度分布を得る段階．

Ｃ；前記第１粒度分布と第２粒度分布との差分に基づいて．前記培養試料中で培養された微生物の粒度分布を得る段階、

Ｄ；前記得られた微生物の粒度分布を出力する段階。

【００１７】前記第１発明と同様の作用効果を有する。

【００１８】

【発明の実施の形態】以下．本発明について、実施形態例を挙げながら具体的に説明する。本発明において、フローサイトメータとは、ａ）試料をシース液で包んで流すことにより流体力学効果による細い試料流を形成させ．ｂ）試料中の粒子を１個ずつ検出部に流し．ｃ）光を照射して粒子から発せられる散乱光や蛍光を検出する装置を言う。光源としては．半導体レーザやアルゴンレーザなどを適宜用いることができる。

【００１９】培養液とは．微生物を培養するための適度な栄養分を含む液体培地をいう。微生物種類を特定しないのであれば一般細菌用培地を、微生物種類を特定するのであればその種類に適した培地を選択的に用いることができる。培養処理とは．微生物を培養するための処理で．所定の温度（例えば３０～３７℃）の恒温器に培養試料を入れ、微生物の培養を促進する処理をいう。

【００２０】また、培養処理前の培養試料を測定とは、

培養処理が行われる前の培養試料中の微生物を測定することである。微生物が増殖しないような低温で培養試料を保存した後に測定することも含む。

＜第１実施形態例＞以下．図面に示す実施形態例に基づいてこの発明を詳述する。これによってこの発明が限定されるものではない。

【００２１】［装置］図１はこの発明のフローサイトメータの検出部と．信号処理装置とを含む構成図である。フローサイトメータの検出部は、シースフローセル１、サンプルノズル２、ビームストッパー５、コレクターレンズ６、ダイクロイックフィルター７．フォトダイオード８．フォトマル９を有している。

【００２２】シースフローセル１は．サンプルノズル２から流れる試料をシース液に包み、シース液に包まれた試料流４を形成する。試料流の粒子４は、図示しないレーザ源からのレーザ光３を照射される。ビームストッパ５は．試料流４を直接透過する光を遮断する。コレクターレンズ６は．粒子４が放出する前方散乱光および前方蛍光を集光する。ダイクロイックフィルター７は．前方散乱光を反射する。フォトダイオード８は、ダイクロイックフィルター７により反射された前方散乱光を検出する。検出された前方散乱光は、アンプを介して信号処理装置１０に入力される。フォトマル９は、ダイクロイックフィルター７を通過した前方蛍光を検出する。フォトダイオード８及びフォトマル９で検出された前方散乱光及び前方蛍光は．アンプを介して信号処理装置１０にそれぞれ入力される。

【００２３】本発明に係る信号処理装置１０は．フォトダイオード８から出力される検出信号を受信し．前方散乱光の発光時間（Ｆｓｃｗ；Ｆｏｒｗａｒｄ　ｓｃａｔｔｅｒ　ｐｕｌｓｅ　ｗｉｄｔｈ）に対する散乱光強度（Ｆｓｃ；Ｆｏｒｗａｒｄｓｃａｔｔｅｒ　ｉｎｔｅｎｓｉｔｙ）の変化を測定する。また．信号処理装置１０は．前方散乱光信号に基づいて粒度分布図を作成し、解析する。図２に．信号処理装置１０に入力される信号の概念図を示す。散乱光強度は、入力信号の強度．すなわちパルス高さに相当する。散乱光の発光時間は．入力信号のパルス幅に相当する。

【００２４】図３は、信号処理装置１０の機能構成を示すブロック図である。信号処理装置１０は、例えばＰＣ（Ｐｅｒｓｏｎａｌ　Ｃｏｍｐｕｔｅｒ）やＷＳ（ＷｏｒｋＳｔａｔｉｏｎ）などの情報端末に設けられ．処理結果を情報端末の出力部に出力する。信号処理装置１０は．粒度分布作成部１０１．記憶部１０２、処理部１０３及び出力制御部１０４を備えている。

【００２５】粒度分布作成部１０１は．フローサイトメータが検出した光信号を受信し、横軸を前方散乱光発光時間．縦軸を前方散乱光発光強度とする粒度分布を受信信号に基づいて作成する。また、粒度分布作成部１０１は．作成した粒度分布を示す粒度分布図を、出力制御部

１０４を介してディスプレイなどの出力部に出力可能である。後述する図６や図７などは、粒度分布作成部１０１により出力される粒度分布図の一例である。

【００２６】記憶部１０２は、作成された粒度分布図を、培養後及び前のそれぞれについて記憶する。また、記憶部１０２は、粒度分布図に基づいて作成される区画データや粒度分布図の解析結果を記憶する。区画データ及び解析結果については後述する。処理部１０３は、粒度分布図を所定の区画に細分し、各区画内のデータ数（以下、単に区画データという）を求める。言い換えれば、区画データとは、粒度分布図上の１区画に含まれる粒子数である。さらに、処理部１０３は、培養前及び後の粒度分布において、同一区画内における区画データの変化分を求める。これにより、試料中の夾雑物を微生物として測定することによる測定誤差を減少することができる。

【００２７】夾雑物による測定誤差について、説明する。フローサイトメータの前方散乱光強度による検出は、培養液中の微生物と同じくらいの大きさの１μｍ以下の夾雑物も検出する。培養処理前でも多数の粒子が検出される（後述する図６参照）。培養処理後に粒子が多数検出されても、それが増殖した微生物なのか最初から混入している夾雑物なのか判断できない。そこで培養処理後の粒度分布から培養処理前の粒度分布を減算し、培養処理によって増加した粒子、すなわち培養された微生物だけを検出する。

【００２８】また、処理部１０３は、各区画データやその変化分に基づいて、所定の方法で粒度分布を解析し、解析結果を表示制御部に出力する。例えば、最も変化分が多い区画を１００％とした場合に、各区画データの変化分の割合を求め、所定の割合以下の区画を表示部分とすることが挙げられる。さらに、処理部１０３は、区画データの変化の度合いを視覚的に表示するために、変化の度合いに応じた色を各区画に設定する。処理部１０３は、培養前及び後の粒度分布についても、同様に区画データの大小に応じた色を各区画に設定してもよい。

【００２９】出力制御部１０４は、処理部１０３により設定された表示色を用い、各区画をディスプレイやプリンタなどの出力部に出力する。後述する図２１～２８は、出力制御部１０４により出力される解析結果の表示例である。縦軸は散乱光強度を、横軸は散乱光の発光時間である。例えば、図２１は、桿菌について、培養前の粒度分布、培養後の粒度分布及び培養により増加した微生物の粒度分布の解析結果をそれぞれ示す。

【００３０】［処理の流れ］次に、信号処理装置１０が行う解析処理の流れを説明する。図４は、信号処理装置１０が行う解析処理の流れの一例を示すフローチャートである。フローサイトメータから検出信号が入力されることにより、以下の処理が開始される。まず、ステップＳ１では、処理部１０３が、所定の初期化処理を行う。具体的には、処理部１０３は、ｎ＝１，ｘ＝１，ｙ＝１とする。ここで、ｎは測定回数を示す変数である。ｘおよびｙは、細分化された粒度分布図の区画のｘ軸方向及びｙ軸方向の位置を示す変数である。

【００３１】ステップＳ２では、粒度分布作成部１０１が、フローサイトメータから検出信号を受信する。ステップＳ３では、粒度分布作成部１０１が、受信した検出信号に基づいて前方散乱光の発光時間に対する発光強度を求め、粒度分布を作成する。さらに粒度分布作成部１０１は、前記作成した粒度分布を記憶部１０２に格納する。粒度分布作成部１０１は、ユーザからの指示に応じてまたは自動的に、粒度分布を記憶部１０２から読み出し、ディスプレイなどに出力可能である。

【００３２】ステップＳ４では、処理部１０３は、所定の区画数に粒度分布図を細分する。例えば、２５６×２５６の区画（１≦ｘ≦２５６，１≦ｙ≦２５６）に細分する。ステップＳ５では、処理部１０３は、各区画毎の区画データを全ての区画について求め、記憶部に格納する。すなわち、処理部１０３は、粒度分布図上の各区画内に含まれる粒子数を、粒度分布に基づいて求める。

【００３３】ステップＳ６では、処理部１０３は、測定回数ｎをインクリメントする。ステップＳ７では、処理部１０３は、測定回数ｎが２であるか否かを判断する。"Ｙｅｓ"と判断すると、２回目の測定を行うためにステップＳ８に移行する。"Ｎｏ"と判断すると、後述するステップＳ９に移行する。培養前と培養後の粒度分布の測定が終了しているので、両者の粒度分布の差から増加した菌数を求めるためである。

【００３４】ステップＳ８では、処理部１０３は、所定時間Ｔが経過するのを待機する。この時間は、検体によって変化するため一概に規定することは難しいが、例えば尿の場合には通常は４時間程度で十分である。ステップＳ９～ステップＳ１４では、前記ステップＳ４で細分した各区画ごとに、区画データの変化分を求め、菌の増加数を決定する処理を行う。

【００３５】まず、ステップＳ９では、処理部１０３は、所定数の区画の中から処理対象の区画を設定する。通常、区画（ｘ＝１，ｙ＝１）から順に処理対象とする。ステップＳ１０では、処理部１０３は、処理対象の区画について、培養後の区画データから培養前の区画データを減算し、区画データの変化分を求める。ステップＳ１１では、処理部１０３は、区画データの変化分が所定値未満であるか否かを判断する。"Ｙｅｓ"と判断すると、ステップＳ１２に移行する。"Ｎｏ"と判断すると、後述するステップＳ１３に移行する。区画データの変化分があまりにも小さい場合には、測定誤差などを考慮して、菌の増加数はないと見なすためである。所定値は、通常、経験的に定められる。

【００３６】ステップＳ１２では、処理部１０３は、処理対象の区画データについて、菌の増加数をゼロに設定

する。また、処理部１０３は、区画と菌の増加数とを対応付けて、記憶部１０２に格納する。ステップＳ１３では、処理部１０３は、処理対象の区画について、区画データの変化分が所定値以上であるので、（菌の増加数）＝（区画データの変化分）とする。前記ステップＳ１２と同様、処理部１０３は、区画と菌の増加数とを対応付けて、記憶部１０２に格納する。

【００３７】ステップＳ１４では、処理部１０３は、全ての区画について、菌の増加数を求めたか否かを判断する。"Ｙｅｓ"と判断すると、ステップＳ１５に移行する。"Ｎｏ"と判断すると、ステップＳ９に戻り、次の区画について菌の増加数を決定する。ステップＳ１５～ステップＳ２０では、培養前後の粒度分布及び増加した菌の解析結果を順に表示する処理が行われる。なお、これら全てを表示する必要はなく、例えば増加した菌についてのみ表示を行っても良い。また、ユーザからの指示に応じて各解析結果を表示することも可能である。

【００３８】まず、ステップＳ１５では、処理部１０３は、培養前、培養後または増加した菌のいずれの粒度分布を解析及び表示対象にするかを決定する。本実施形態例では、培養前、培養後、増加した菌の順に表示するものとする。ステップＳ１６では、処理部１０３は、区画データが最大である区画の区画データを１００％とした場合の、各区画データの割合を求める。解析対象が増加した菌の場合、菌の増加数が最大である区画の菌数を１００％とした場合の、各区画の菌の増加数の割合を求める。

【００３９】ステップＳ１７では、処理部１０３は、区画データまたは菌の増加数が所定の割合以下の区画を、表示部分に決定する。これは、全ての区画を表示対象とすると、粒度分布が見にくくなり増殖した菌の特性が判別しにくくなるからである。後述する図２１～図４４は、区画データまたは菌の増加数の割合が１０％以下の部分を表示部分とした場合の解析結果を示している。

【００４０】ステップＳ１８では、処理部１０３は、区画データまたは菌の増加数の割合を予め定めておいたレベルに分け、レベル毎に異なる所定の表示色を表示部分内の各区画について設定する。後述する図２１～図４４は、０～２％、２～４％、４～６％、６～８％、８～１０％の５つのレベル毎に異なる色を用いて表示した解析結果の例である。

【００４１】ステップＳ１９では、出力制御部１０４が、表示部分を設定された表示色を用いて出力部に出力する。これにより、図２１～２８に例示する解析結果が出力部に表示される。ステップＳ２０では、処理部１０３は、培養前、培養後または培養後－培養前の全てについて、解析結果を表示したか否かを判断する。"Ｙｅｓ"と判断すると、処理を終了する。"Ｎｏ"と判断すると、再び前記ステップＳ１５に戻り、表示されていない解析結果を表示する。

【００４２】［実施例］前記フローサイトメータ及び信号処理装置を用いて行った、試料中の微生物の測定について説明する。

（１）微生物の数の測定

（１－１）培養及び測定

検体としては尿を用いた。尿中細菌の検査は、膀胱炎や腎盂腎炎などの尿路感染症の診断のために、臨床検査としてひろく行われている。

【００４３】尿中の細菌培養液として、一般細菌用液体培地であるハートインフィージョンブイヨン（ニッスイ製）を用いた。使用方法としては、取扱い説明に従い、加温融解したものを高圧蒸気滅菌してから使用した。まず、培養液２ｍｌをいれた試験管２本を用意した。尿検体１００μｌを各試験管に添加し、撹拌し、培養試料２本を準備した。培養試料の１本を培養処理せずに、後述するようにフローサイトメータで微生物数を測定した。もう１本の培養試料を恒温機に入れて３７℃で４時間培養した後、フローサイトメータで同様に微生物数を測定した。

【００４４】フローサイトメータによる微生物数の測定は、次のように行った。フローサイトメータで分析する試料の量は、０．８μｌとした。フローサイトメータにより前方散乱光を検出し、検出光を信号処理装置１０に入力し、前方散乱光発光時間に対する前方散乱光強度を測定した。培養処理された培養試料の測定結果を第１粒度分布、培養処理前の培養試料の測定結果を第２粒度分布とし、第１粒度分布と第２粒度分布との差を求めて培養試料の最終的な粒度分布を求めた。

【００４５】図６及び図７は、検体の第１及び第２粒度分布の測定結果を示す。横軸は散乱光発光時間Ｆｓｃｗ、縦軸は散乱光強度Ｆｓｃである。図６及び図７に示す粒度分布の縦軸の１００ｃｈは、粒子径にして約１μｍに相当する。図６及び図７の粒度分布から、２５６７６個もの粒子が増殖したことが分かった。増殖した粒子数は、培養の前後におけるデータ数（各粒度分布におけるプロット数）の差から求めた。

【００４６】（１－２）従来の測定法との精度比較

次に、培養前後の粒度分布から求めた微生物量と、従来の寒天平板培地を用いて培養及び測定される微生物量との比較を行った。前記（１－１）で述べたのと同様に試料を準備して培養し、粒度分布の測定を行った。測定に基づいて微生物数を求めた（以下、本法という）。

【００４７】比較対象とする寒天平板培地による培養及び測定は、次のように行った。まず、寒天平板培地として、尿中微生物検査で通常用いられるウリカルトＥ（Ｏｒｉｏｎ　Ｄｉａｇｎｏｓｔｉｃａ製）を用いた。この培地はＣＬＥＤ培地とマッコンキー培地とエンテロコッカス培地とから構成されている。取扱い説明書に従い培地表面に尿を塗抹し、３７度で２４時間培養を行なった。判定はコロニー数の密度を目視にて所定の対照表と

比較し、尿中の菌数を求めた。

【００４８】前記ウリカルトＥ法と本法とを４０検体について行った。測定結果を図８に示す。図８は、ウリカルトＥ法及び本法による菌数の測定結果を示している。ウリカルトＥ法では、細菌数10^5／ｍｌ以上を陽性（細菌尿）と判定し、細菌数10^4／ｍｌを陽性の疑いがある判定保留とする。そこで、ウリカルトＥ法で10^4個／ｍｌ以上となった１６検体について、本法の測定結果と比較した。本法による測定結果は、いずれの１６検体についても10^4個／ｍｌ以上という同等の測定結果を示した。また、測定された値の差は、１桁の範囲内であった。このことから、ウリカルトＥ法と本法との良好な相関関係が示されたと言える。

【００４９】次に、ウリカルトＥ法で陰性である一方、本法では高値の陽性結果となった検体（図８中矢印Ａ）について、再検査を行なった。すると、ウリカルトＥの培地表面に溶けているところがあった。これは細菌コロニーが密集しすぎて起こったものである。このことから、この検体については、ウリカルトＥ法において細菌が増殖しているのにコロニーが観察されないため、陰性と誤判定されたと考えられる。

【００５０】次にウリカルトＥ法に比べ本法が低値となった２検体（図８中矢印Ｂ）について、検討した。両検体の培養後の粒度分布図（図９、図１１）は、ともに散乱光発光時間が長いほうに広がる粒度分布を示している。この粒度分布は培養前の粒度分布図（図１０、図１２）には見られないので、培養によって増殖した菌の粒度分布である。そこでこれらの培養試料を顕微鏡でさらに詳細に調べた。これらの検体では、いずれも菌が連鎖して増殖していることが観察された。さらに菌種を調べると、図９及び図１０で示される菌は連鎖球菌のＥｎｔｅｒｏｃｏｃｃｕｓ　ｅｒｏｇｅｎｅｓであり、図１１及び図１２で示される菌は連鎖桿菌のＰｓｅｕｄｏｍｏｎａｓであった。

【００５１】以上から、ウリカルトＥ法と本法との測定結果が一致しなかった原因が次のように考えられる。連鎖桿菌及び連鎖球菌は、鎖状に連なりながら増殖していく。ところが、フローサイトメータは、複数の菌が連鎖した鎖状の固まりを一つの粒子として検出する。そこで、フローサイトメータは、菌の増殖が起こっていても、連鎖しているためにに実際の増加分を検出できない。

【００５２】連鎖桿菌及び連鎖球菌は、長く連なった形状であるので、散乱光強度はほとんど変わらないが、散乱光の発光時間が連鎖した菌の長さに応じて長くなる。従って、散乱光発光時間が長い信号を監視することにより、信号強度だけでは見逃してしまう連鎖桿菌及び連鎖球菌の増殖を検出可能である。具体的には、散乱光発光時間が例えば９０ｃｈ以上の領域に所定数以上の菌が検出される場合、連鎖桿菌または連鎖球菌が存在している陽性であると判定をすることができる。さらに、その領域の信号については、散乱光発光時間の長さに応じ、連鎖した菌数に対応させる係数を乗じることにより、増殖した微生物数を鑑定可能である。

【００５３】以上の検討結果より、本法は従来法であるウリカルトＥ法と良好な相関関係を有し、しかも従来より大幅に短い培養時間で良好な測定結果を提供出来ることが分かった。培養液の種類を選択性培地にすることで、上記と同じ操作にて、微生物の種類を特定することも可能である。培養時間が短時間で済むので、培養条件を変えて微生物を詳細に検討することが短時間で可能となる。

【００５４】さらに、連鎖桿菌及び連鎖球菌以外の各種の微生物について、増殖形態と粒度分布図との関係を調べてみた。連鎖桿菌及び連鎖球菌以外に菌が集団となって増殖する菌としてブドウ球菌がある。２種類のブドウ球菌を試料としてそれぞれ前記（１－１）と同様に培養し、フローサイトメータにより散乱光を検出して信号処理装置による粒度分布の測定を行なった。その粒度分布図が図１７、図１８である。前記の連鎖菌とは異なり、粒度分布は散乱光強度の大きいほうへ細長く伸びる粒度を示す。ブドウ球菌は増殖によって菌が房のように集まりながら増えてゆく。従って、菌の増殖に従い、菌の集団の径が大きくなり、散乱光強度も大きくなる。

【００５５】菌が増殖しても集団とはならない菌である桿菌についても同様に粒度分布を調べてみた。その粒度分布図を図１５、図１６に示す。散乱光信号の強度や発光時間が大きいほうに広がるような粒度分布は示されていない。これは菌が増殖しても菌どうしがばらばらの状態であるので、散乱光信号の強度や発光時間が変わることなく一定値以下のところに現れることを示している。

【００５６】以上の検討により、フローサイトメータでは菌の集団を一つの粒子として捉えるために、連鎖桿菌、連鎖球菌及びブドウ球菌について実際の増殖数より低く計測していることがわかった。また、連鎖桿菌、連鎖球菌、ブドウ球菌及び桿菌は、増殖形態が異なるために粒度分布が異なり、増殖した菌の粒度分布に基づいて、菌の種類を特定可能であることが分かった。なかでも、連鎖桿菌及び連鎖球菌は、散乱光強度が単独の桿菌とあまり変わらないので、散乱光強度を測定するだけでは判別ができない。散乱光信号の強度と発光時間との２つのパラメータを測定することによって、ブドウ球菌、連鎖球菌、連鎖桿菌及び桿菌を区別することが可能となる。図４５は、前記４種の菌による粒度分布の違いを模式的に示したものである。

【００５７】具体的には、例えば散乱光強度が９０ｃｈ以下で散乱光の発光時間が９０ｃｈ以上の領域に菌が所定数以上検出される場合、連鎖桿菌が存在していると警報を出来ことが考えられる。また、散乱光強度が５０ｃｈ以上で散乱光の発光時間が８０ｃｈ以下の領域に菌が

所定数以上検出される場合は、ブドウ球菌が存在していると警報を出すことが考えられる。連鎖桿菌もブドウ球菌も増殖に従って粒度分布が延びてゆくので、判定を領域の個数だけでは粒度分布の形状をも監視することが可能である。

【００５８】さらに、集団の菌数によって粒度分布が変化するので、その領域の信号については、散乱光の強度もしくは発光時間の大きさに応じて集まった菌数に対応させた係数を乗じることにより、増殖した菌数自体を推定することも可能である。酵母様真菌についても粒度分布を測定したのでその結果を示す。図１３及び図１４は、酵母様真菌の粒度分布図を示している。散乱光強度＊が２５０ｃｈを超えたところに信号が現れるため、この図面に粒度が現れていない。酵母様真菌は大きさが３～５μｍと大きいため、散乱光強度も強くなるからである。

【００５９】（２）微生物の分類

本実施形態例に係る信号処理装置により測定結果の解析を行い、微生物の分類を行ったので、測定結果及び解析結果について説明する。培養対象としては下記の細菌を用いた。下記の細菌は、尿中に検出される細菌であり、検体に尿を用いて細菌検査を行う場合に検査対象となる細菌である。

【００６０】

桿菌（１）	Ｅｓｃｈｅｒｉｃｈｉａ　ｃｏｌｉ
桿菌（２）	Ｐｓｅｕｄｏｍｏｎａｓ　ａｅｒｕｇｉｎｏｓａ
ブドウ球菌（１）	Ｓｔａｐｈｙｌｏｃｏｃｏｕｓ　ａｕｒｅｕｓ
ブドウ球菌（２）	Ｓｔａｐｈｙｌｏｃｏｃｏｕｓ　ｅｐｉｄｅｒｍｉｄｉｓ
連鎖球菌（１）	Ｅｎｔｅｒｃｏｃｏｃｕｓ　ｆａｅｃａｌｉｓ
連鎖球菌（２）	Ｓｔｒｅｐｔｏｃｏｃｏｕｓ　ａｇａｌａｃｔｉａｅ
連鎖桿菌	Ｐｓｅｕｄｏｍｏｎａｓ
酵母様真菌	Ｃａｎｄｉｄａ　ｇｌａｂｒａｔａ

これらの細菌を、前記（１－１）と同様に培養し、フローサイトメータにより散乱光を検出して信号処理装置による粒度分布の測定を行った。ただし、培養時間は４時間ではなく２時間として実験を行った。図１５～２０は、各細菌の培養前及び培養後の粒度分布を示す。図１５は桿菌（１）、図１６は桿菌（２）、図１７はブドウ球菌（１）、図１８はブドウ球菌（２）、図１９は連鎖球菌（１）、図２０は連鎖球菌（２）についての粒度分布である。各図において（ａ）は培養前の粒度分布を、（ｂ）は培養後の粒度分布を示している。また、連鎖桿菌の粒度分布は前記図１１及び１２に示すとおりである。酵母様真菌の粒度分布は、前記図１３及び１４に示すとおりである。

【００６１】前記図１５～２０の各図において、図（ａ）と図（ｂ）とを比較することにより、粒度分布図上のどの領域で細菌が増加しているかが分かる。しかし、著しい変化がない場合、前記変化が分かりにくい。そこで、前述のように、粒度分布図の区画毎に培養の前後における粒度分布の変化分を求め、変化の割合が所定範囲の区画を表示する解析処理を行った。

【００６２】解析結果を図２１～図４４に示す。解析結果は、前記粒度分布のグラフを２５６×２５６の区画に分割し、各区画における粒子数を割り出し、最も多い粒子数を１００％とした場合の１０％以下の区画を、所定のレベル毎に色分けして表示したグラフである。図２１、２２及び図２３は、桿菌（１）について、培養前、培養後及びその変化分の粒度分布をそれぞれ示す解析結果である。また、図２４、２５及び図２６は、桿菌（２）について、培養前、培養後及びその変化分の粒度分布をそれぞれ示す解析結果である。桿菌（１）の散乱光強度は、おおむね１０～８０ｃｈの範囲にある。桿菌（２）の散乱光強度は、０～２０ｃｈの範囲にある。また、散乱光の発光時間は、桿菌（１）でおおむね４０～９０ｃｈの範囲であり、桿菌（２）で３０～７０ｃｈの範囲と短い。桿菌の種類によって粒度分布の位置に少し違いはあるが、粒度分布はあまり広がらずにまとまっている。すなわち桿菌は増殖しても集団とはならないことが示されている。また、桿菌（２）は粒子径の小さい小型桿菌であることが示されている。

【００６３】図２７、２８及び２９は、ブドウ球菌（１）について、培養前、培養後及びその変化分の粒度分布をそれぞれ示す解析結果である。また、図３０、３１及び３２は、ブドウ球菌（２）について、培養前、培養後及びその変化分の粒度分布をそれぞれ示す解析結果である。培養対象がブドウ球菌であるため、増殖した菌の散乱光強度が広い範囲に渡っている。

【００６４】例えば、ブドウ球菌（１）では、増殖した菌の散乱光強度は、主に２０～１９０ｃｈの範囲に渡っている。ブドウ球菌（２）では、増殖した菌の散乱光強度は、主に０～１６０ｃｈの範囲に渡っている。しかし、散乱光の発光時間は４０～９０ｃｈの範囲にあり、散乱光強度が大きくなるにつれて大きくなっている。すなわち、菌が、鎖状ではなく房状に増殖していることが示されている。

【００６５】図３３、３４及び３５は、連鎖球菌（１）について、培養前、培養後及びその変化分の粒度分布をそれぞれ示す解析結果である。また、図３６、３７及び３８は、連鎖球菌（２）について、培養前、培養後及びその変化分の粒度分布をそれぞれ示す解析結果である。培養対象が連鎖球菌であるため、増殖した菌の散乱光強

度は主に０～５０ｃｈの小さい範囲にまとまっている。しかし、散乱光の発光時間は、連鎖球菌（１）では４０～２２０ｃｈ．連鎖球菌（２）では３０～１７０ｃｈの範囲に、散乱光強度の大きさにかかわらず広がっている。すなわち．増殖した菌は、小径ではあるが連鎖して長さが長くなっていることが示されている。

【００６６】図３９、４０及び４１は．連鎖桿菌について．培養前、培養後及びその変化分の粒度分布をそれぞれ示す解析結果である。培養対象が連鎖桿菌であるため．増殖した菌の散乱光強度は主に３０～６０ｃｈの小さい範囲にまとまっている。しかし．散乱光の発光時間は．６０～１８０ｃｈの範囲に散乱光強度の大きさにかかわらず広がっている。すなわち、小径な菌が連鎖して増殖していることが示されている。連鎖球菌、連鎖桿菌はいずれも菌の連鎖度合いに従って散乱光発光時間がおおきくなってゆく。しかし散乱光強度は各菌の径によるため．連鎖桿菌の方が連鎖球菌より径が大きいため、散乱光強度も大きくなる。

【００６７】図４２、４３及び４４は．酵母様真菌について．培養前．培養後及びその変化分の粒度分布をそれぞれ示す解析結果である。培養対象が酵母様真菌であるため．増殖した菌の散乱光強度は図では２４０ｃｈ近辺にあるが、これは２４０ｃｈ以上の大きい信号が含まれている。また．発光時間は６０～１５０ｃｈの範囲に渡っている。

【００６８】以上から、ユーザは、培養により増殖した菌の解析結果に基づいて．桿菌、ブドウ球菌、連鎖球菌．連鎖桿菌及び酵母様真菌の５つの分類のいずれに、増殖した菌が該当するか類推可能である。また．解析結果に基づいて．増殖した菌が前記５つの分類のいずれに該当するかを信号処理装置１０により判定することも可能である。例えば、前記５つの分類のそれぞれに属する粒度分布上の区画領域を予め定めておき、各区画領域の信号数に応じて．分類結果を表すことが考えられる。又．粒度分析のピーク位置や分布幅を解析して菌を分類することも考えられる。

【００６９】さらに、検体が尿である場合、尿中の桿菌及び連鎖桿菌はそのほとんどがグラム陰性であり．尿中のブドウ球菌及び連鎖球菌はそのほとんどがグラム陽性であることが知られている。従って．前記増殖した菌の解析結果に基づいて菌の分類を類推し．グラム陽性／陰性までも推定することが可能である。

【００７０】

【発明の効果】本発明を利用することにより、フローサイトメータを用い、微生物を短時間の培養によって簡単に測定することができ、測定結果に及ぼす夾雑物の影響を減少して正確な測定を期することができる。さらに、フローサイトメータが検出する散乱光の発光時間及び信号強度から、微生物の分類の概要を類推することができ．検査に要する時間を短縮することができる。

【図面の簡単な説明】

【図１】フローサイトメータの検出部の構成を示す説明図。

【図２】フローサイトメータに入力される信号の説明図。

【図３】信号処理装置の機能構成を示すブロック図。

【図４】信号処理装置が行う解析処理の流れを示すフローチャート（前半）。

【図５】信号処理装置が行う解析処理の流れを示すフローチャート（後半）。

【図６】培養処理後の粒度分布図（尿）。

【図７】図３の試料培養処理前の粒度分布図。

【図８】ウリカルトＥ法と本法との測定結果。

【図９】連鎖球菌の培養処理後の粒度分布図。

【図１０】図６の連鎖球菌の培養処理前の粒度分布図。

【図１１】連鎖桿菌の培養処理後の粒度分布図。

【図１２】図１１の連鎖桿菌の培養処理前の粒度分布図。

【図１３】酵母様真菌の培養処理後の粒度分布図。

【図１４】図１０の酵母様真菌の培養処理前の粒度分布図。

【図１５】（ａ）培養前の桿菌（１）の粒度分布
（ｂ）培養後の桿菌（１）の粒度分布

【図１６】（ａ）培養前の桿菌（２）の粒度分布
（ｂ）培養後の桿菌（２）の粒度分布

【図１７】（ａ）培養前のブドウ球菌（１）の粒度分布
（ｂ）培養後のブドウ球菌（１）の粒度分布

【図１８】（ａ）培養前のブドウ球菌（２）の粒度分布
（ｂ）培養後のブドウ球菌（２）の粒度分布

【図１９】（ａ）培養前の連鎖球菌（１）の粒度分布
（ｂ）培養後の連鎖球菌（１）の粒度分布

【図２０】（ａ）培養前の連鎖球菌（２）の粒度分布
（ｂ）培養後の連鎖球菌（３）の粒度分布

【図２１】桿菌（１）の粒度分布の解析結果（培養前）。

【図２２】桿菌（１）の粒度分布の解析結果（培養後）。

【図２３】桿菌（１）の粒度分布の解析結果（培養後－前）。

【図２４】桿菌（２）の粒度分布の解析結果（培養前）。

【図２５】桿菌（２）の粒度分布の解析結果（培養後）。

【図２６】桿菌（２）の粒度分布の解析結果（培養後－前）。

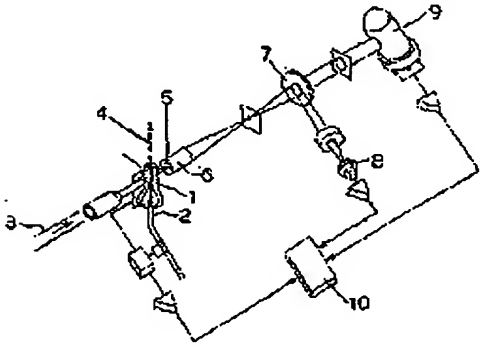
【図２７】ブドウ球菌（１）の粒度分布の解析結果（培養前）。

【図２８】ブドウ球菌（１）の粒度分布の解析結果（培養後）。

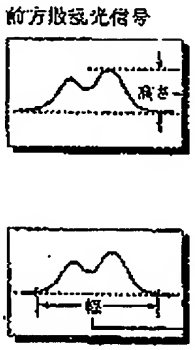
【図２９】ブドウ球菌（１）の粒度分布の解析結果（培

養後－前）。
【図３０】ブドウ球菌（２）の粒度分布の解析結果（培養前）。
【図３１】ブドウ球菌（２）の粒度分布の解析結果（培養後）。
【図３２】ブドウ球菌（２）の粒度分布の解析結果（培養後－前）。
【図３３】連鎖球菌（１）の粒度分布の解析結果（培養前）。
【図３４】連鎖球菌（１）の粒度分布の解析結果（培養後）。
【図３５】連鎖球菌（１）の粒度分布の解析結果（培養後－前）。
【図３６】連鎖球菌（２）の粒度分布の解析結果（培養前）。
【図３７】連鎖球菌（２）の粒度分布の解析結果（培養＊後）。
【図３８】連鎖球菌（２）の粒度分布の解析結果（培養後－前）。
【図３９】連鎖桿菌の粒度分布の解析結果（培養前）。
【図４０】連鎖桿菌の粒度分布の解析結果（培養後）。
【図４１】連鎖桿菌の粒度分布の解析結果（培養後－前）。
【図４２】酵母様真菌の粒度分布の解析結果（培養前）。
【図４３】酵母様真菌の粒度分布の解析結果（培養後）。
【図４４】酵母様真菌の粒度分布の解析結果（培養後－前）。
【図４５】ブドウ球菌、連鎖球菌、連鎖桿菌及び桿菌の菌種による粒度分布の違いを示す模式図。

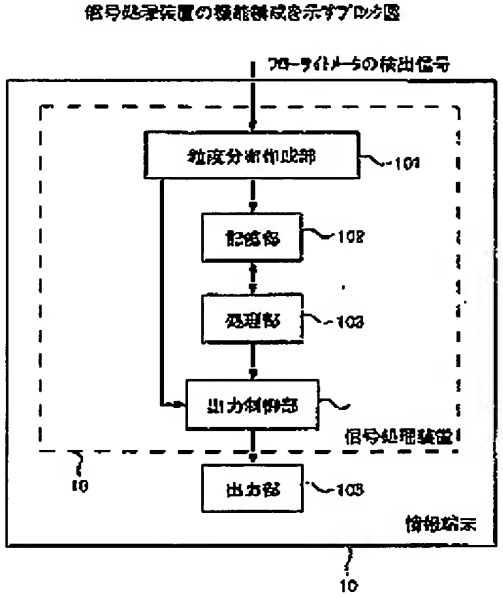
【図１】



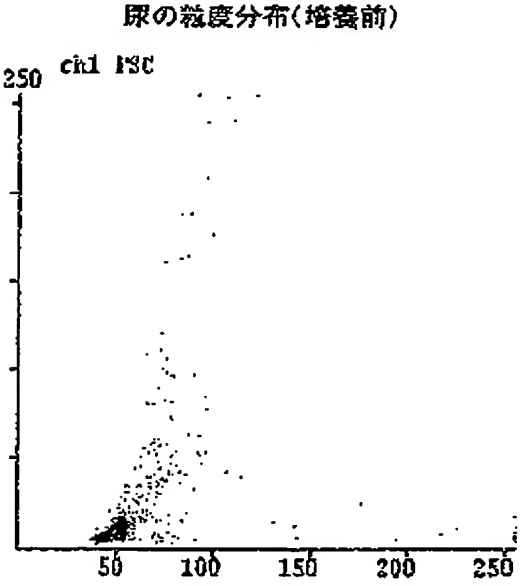
【図２】



【図３】

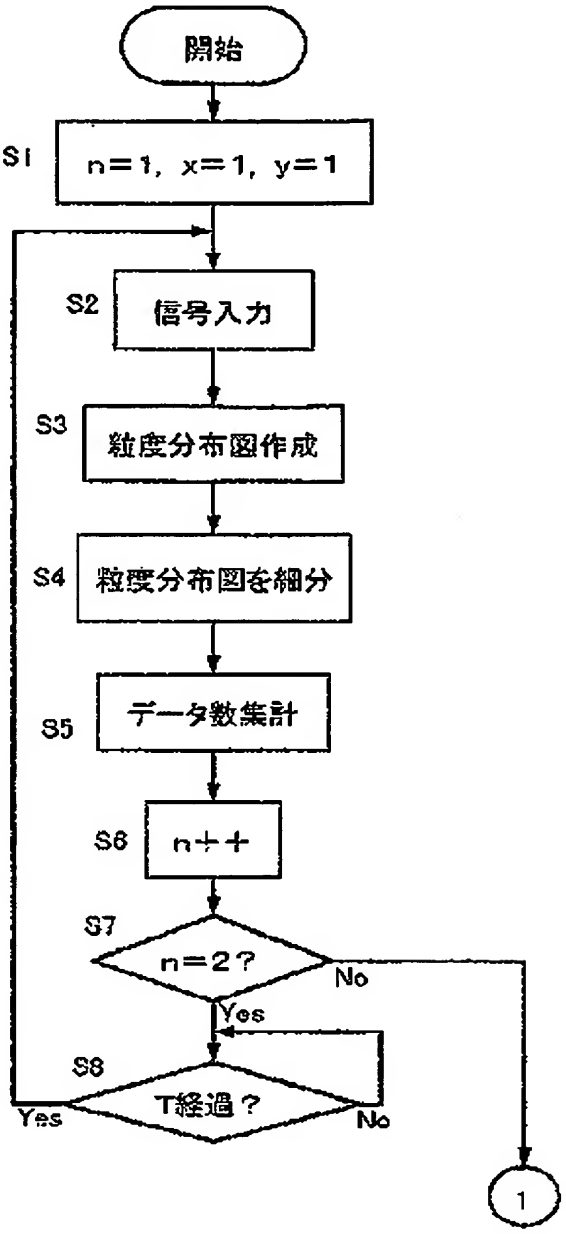


【図６】



【図４】

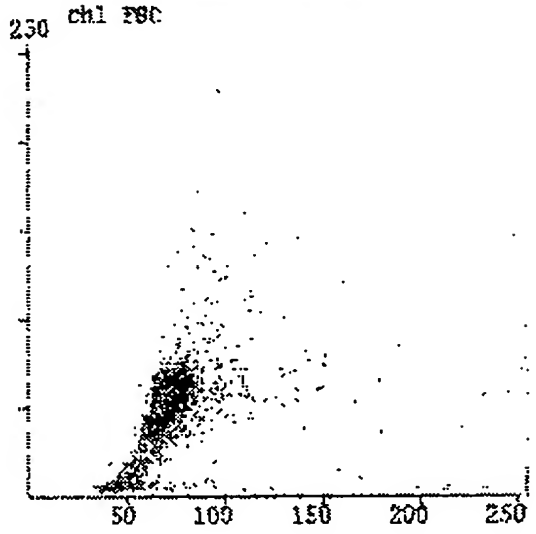
信号処理装置が行う処理の流れ(１)



【図７】

図面代用写真

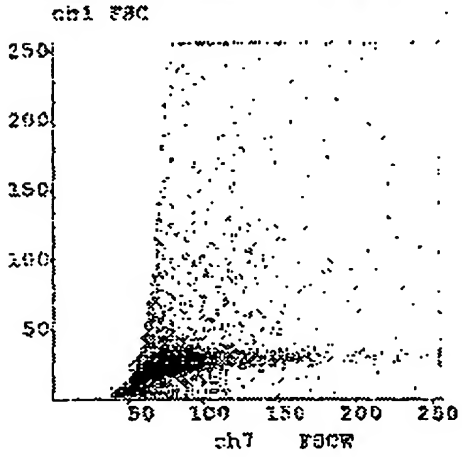
腸の粒度分布(培養後)



【図９】

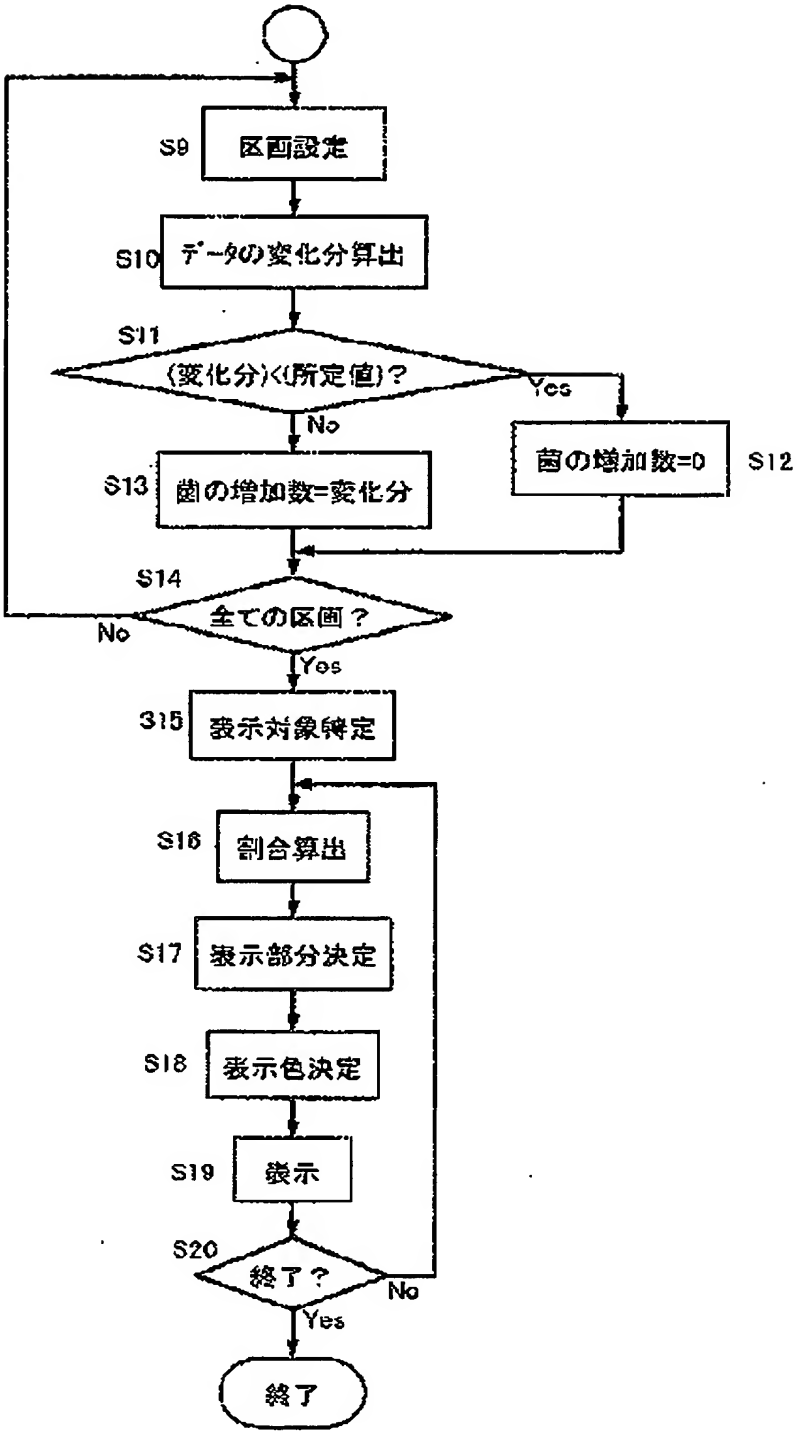
図面代用写真

連鎖球菌の粒度分布(培養後)



【図５】

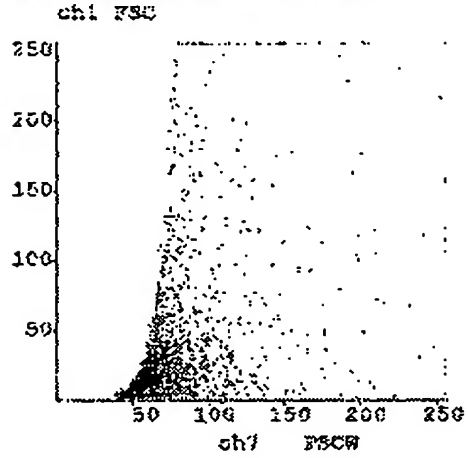
信号処理装置が行う処理の流れ(2)



【図１０】

図面代用写真

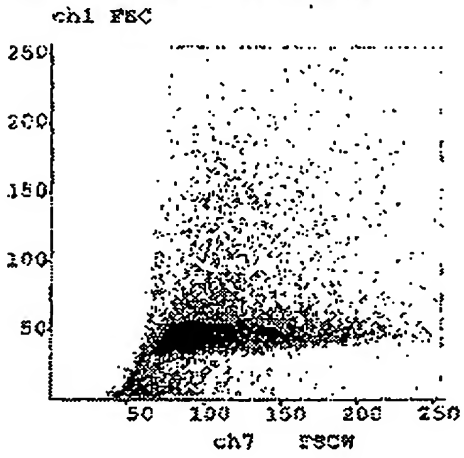
連鎖球菌の粒度分布(増殖前)



【図１１】

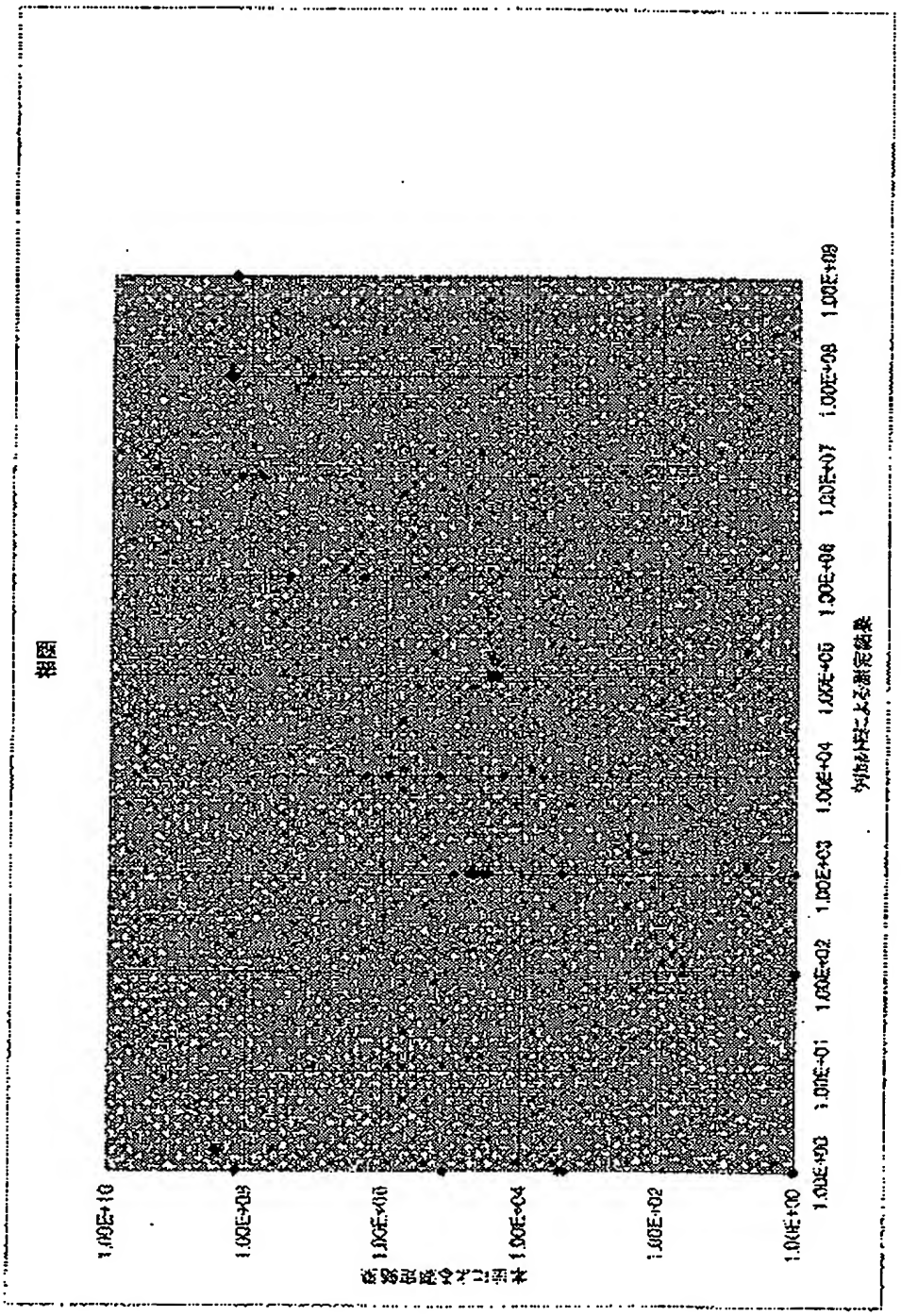
図面代用写真

連鎖球菌の粒度分布(増殖後)



【図８】

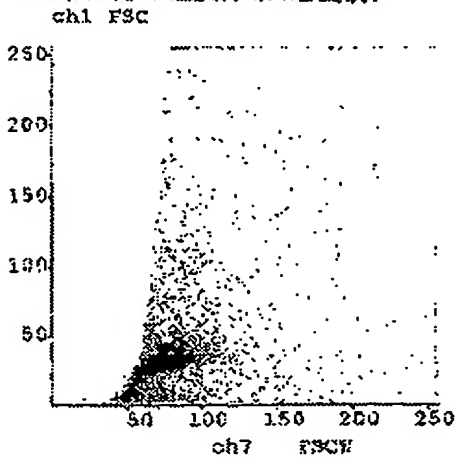
図面代用写真


特図
1.00E+10
1.00E+08
1.00E+06
1.00E+04
1.00E+02
1.00E+00
1.00E+00
1.00E-01
1.00E-02
1.00E+03
1.00E+04
1.00E+05
1.00E+06
1.00E+07
1.00E+08
1.00E+09

【図１２】

図面代用写真

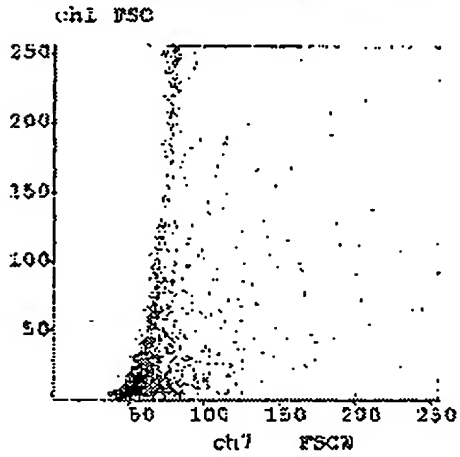
連鎖球菌の粒度分布（培養前）



【図１３】

図面代用写真

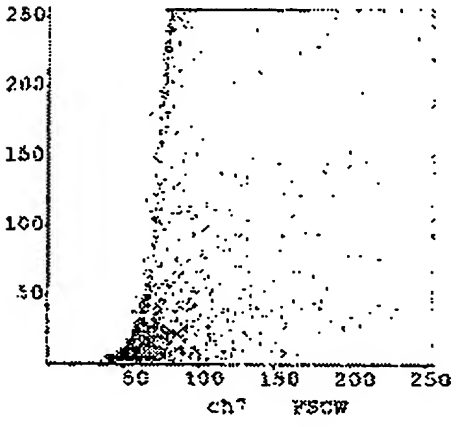
酵母様真菌の粒度分布（培養後）



【図１４】

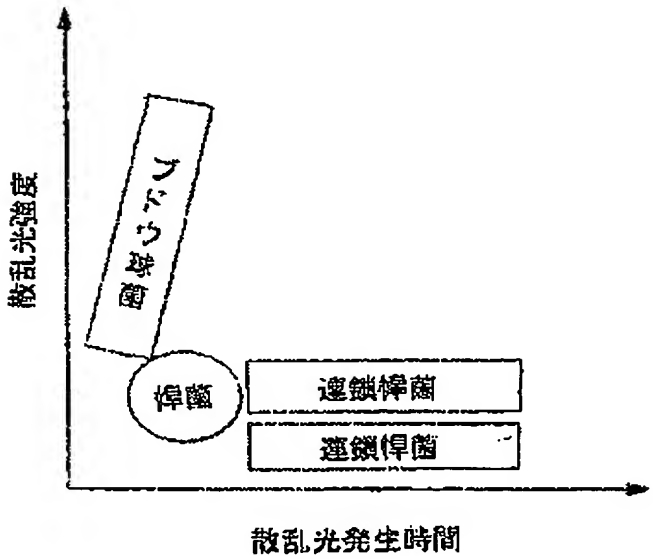
図面代用写真

酵母様真菌の粒度分布（培養前）

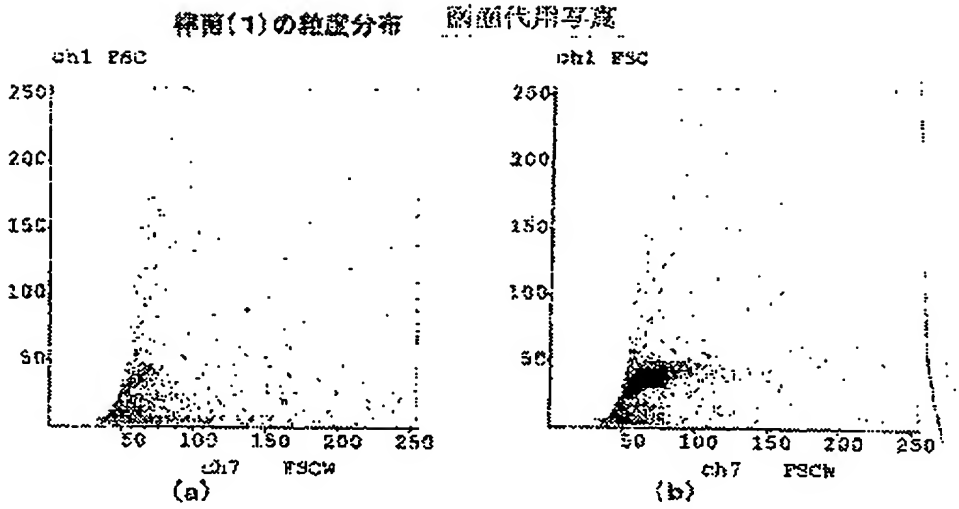


【図４５】

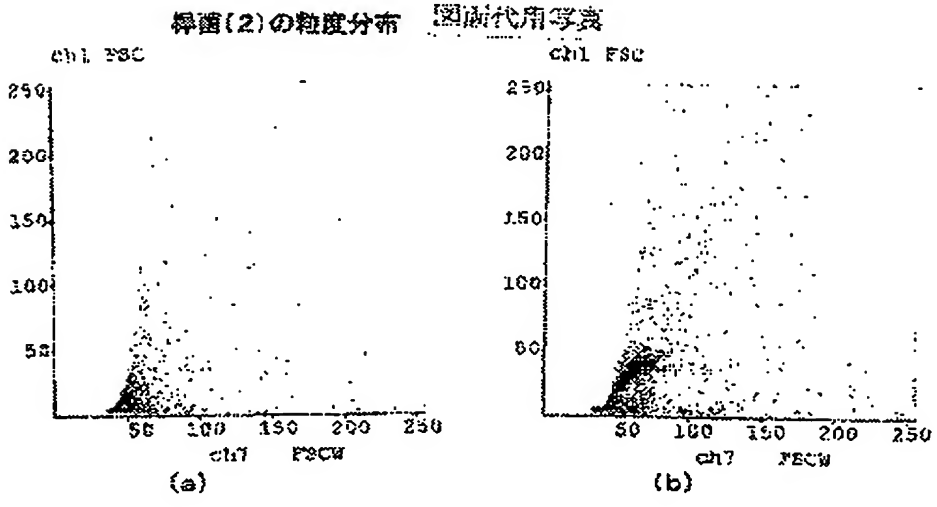
面積による粒度分布の違いを示す模式図



【図１５】

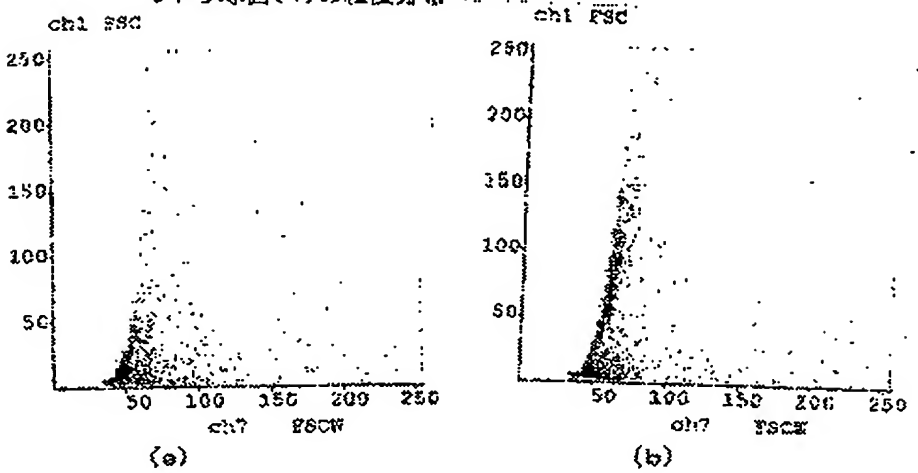

緑茵(1)の粒度分布 図面代用写真
ch1 FSC
250
200
150
100
50
50 100 150 200 250
ch7 FSCW
(a)
ch1 FSC
250
200
150
100
50
50 100 150 200 250
ch7 FSCW
(b)

【図１６】


緑茵(2)の粒度分布 図面代用写真
ch1 FSC
250
200
150
100
50
50 100 150 200 250
ch7 FSCW
(a)
ch1 FSC
250
200
150
100
50
50 100 150 200 250
ch7 FSCW
(b)

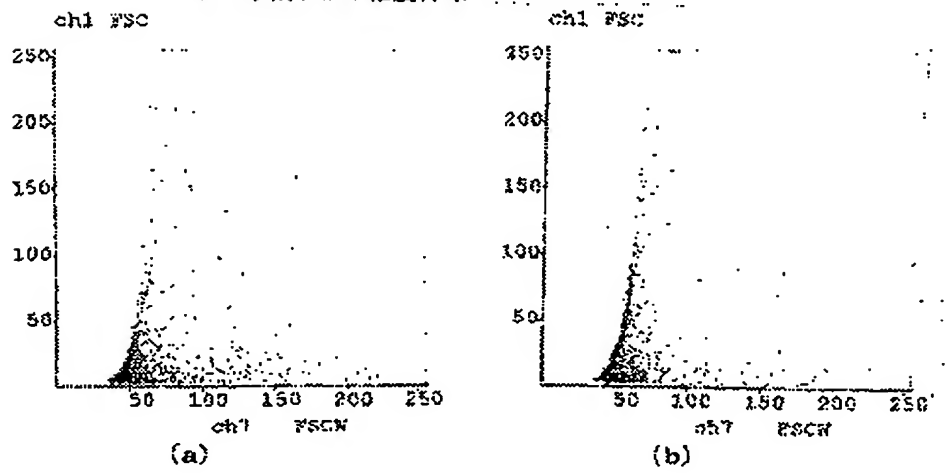
【図１７】

ブドウ球菌(1)の粒度分布　図面代用写真



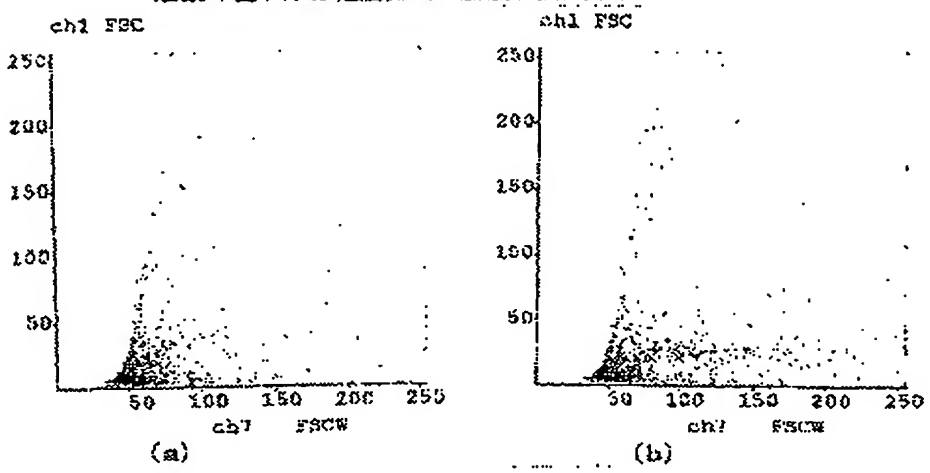
【図１８】

ブドウ球菌(2)の粒度分布　図面代用写真



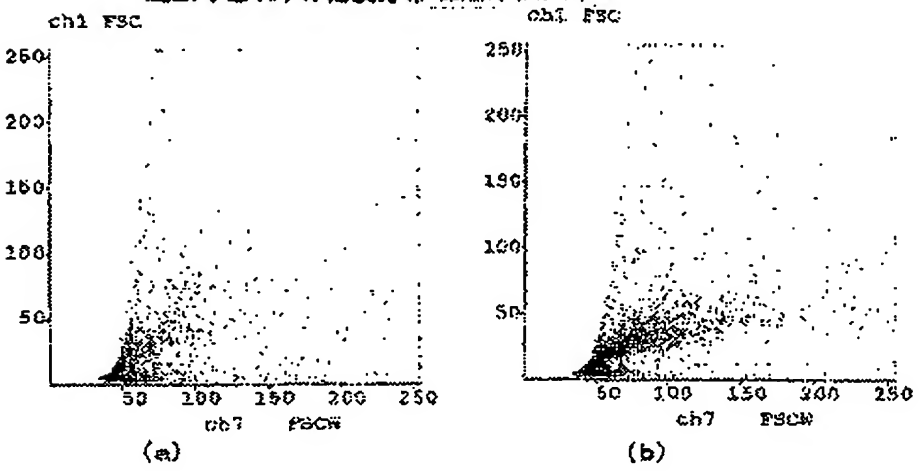
【図１９】

連鎖球菌(1)の粒度分布 図面代用写真



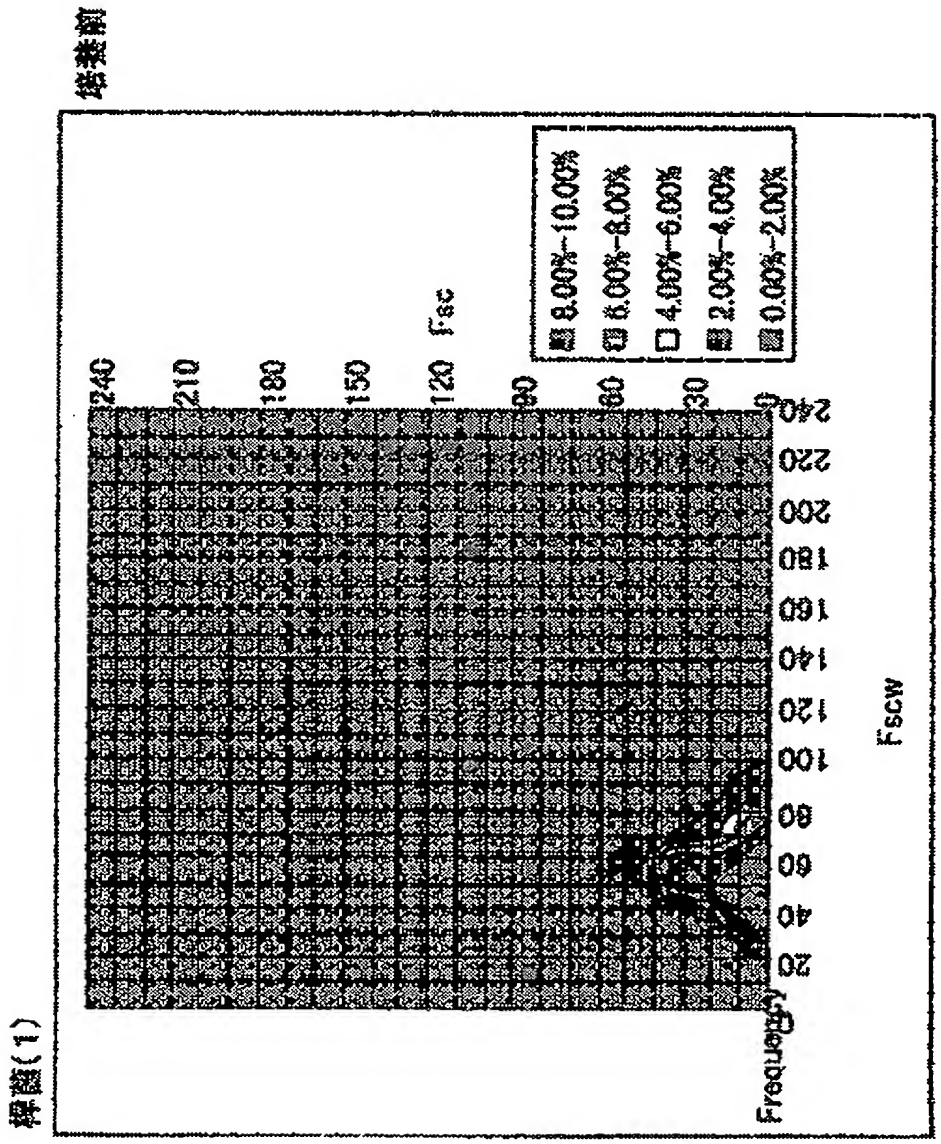
【図２０】

連鎖球菌(2)の粒度分布 図面代用写真



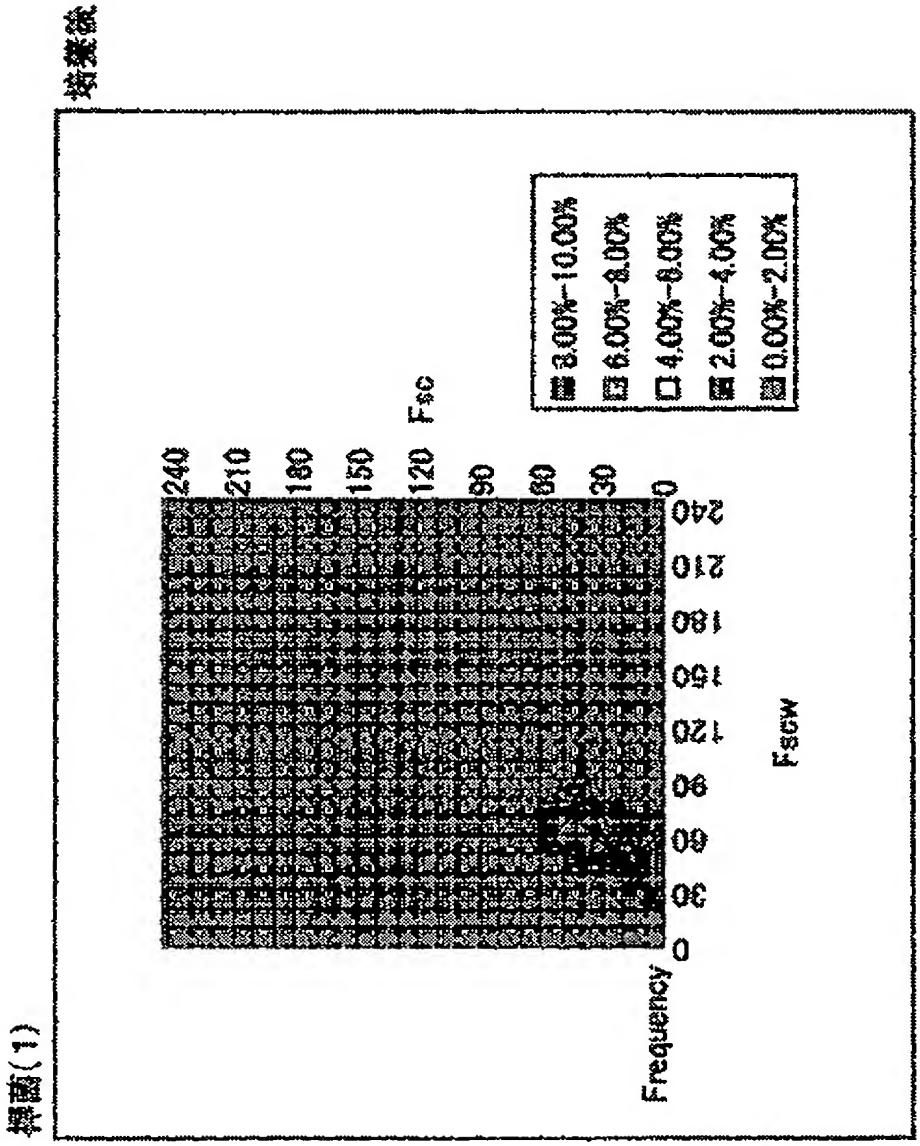
【図２１】

図面代用写真（カラー）



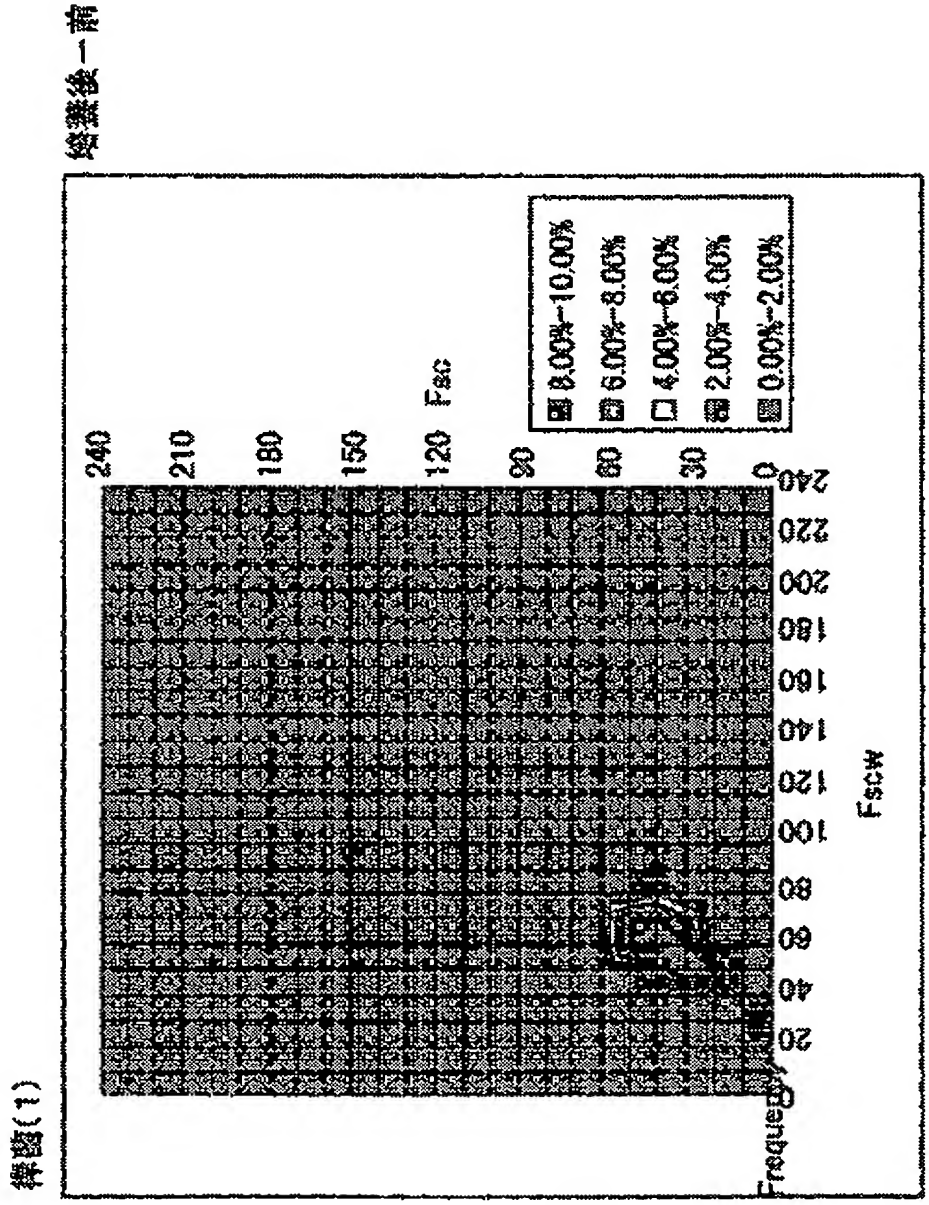
【図２２】

図面代用写真（カラー）



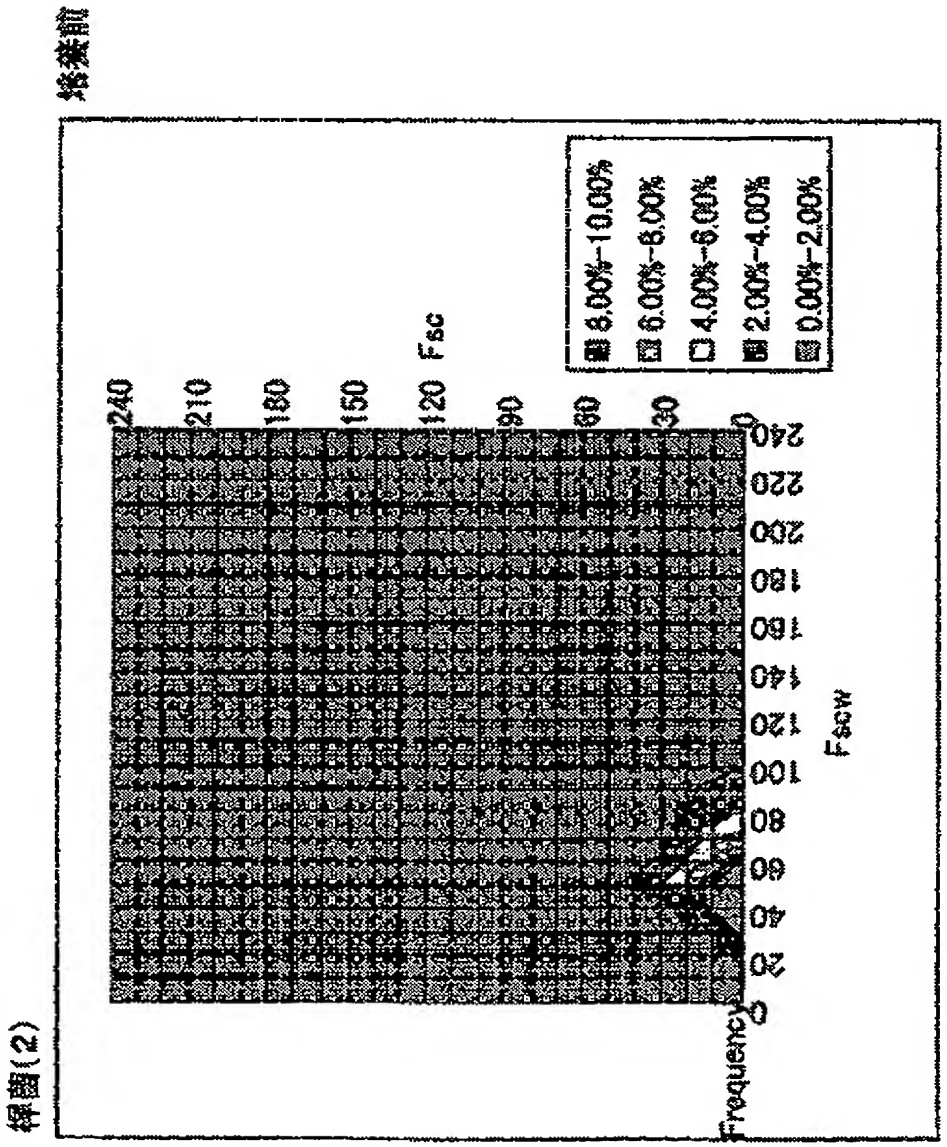
【図２３】

図面代用写真（カラー）



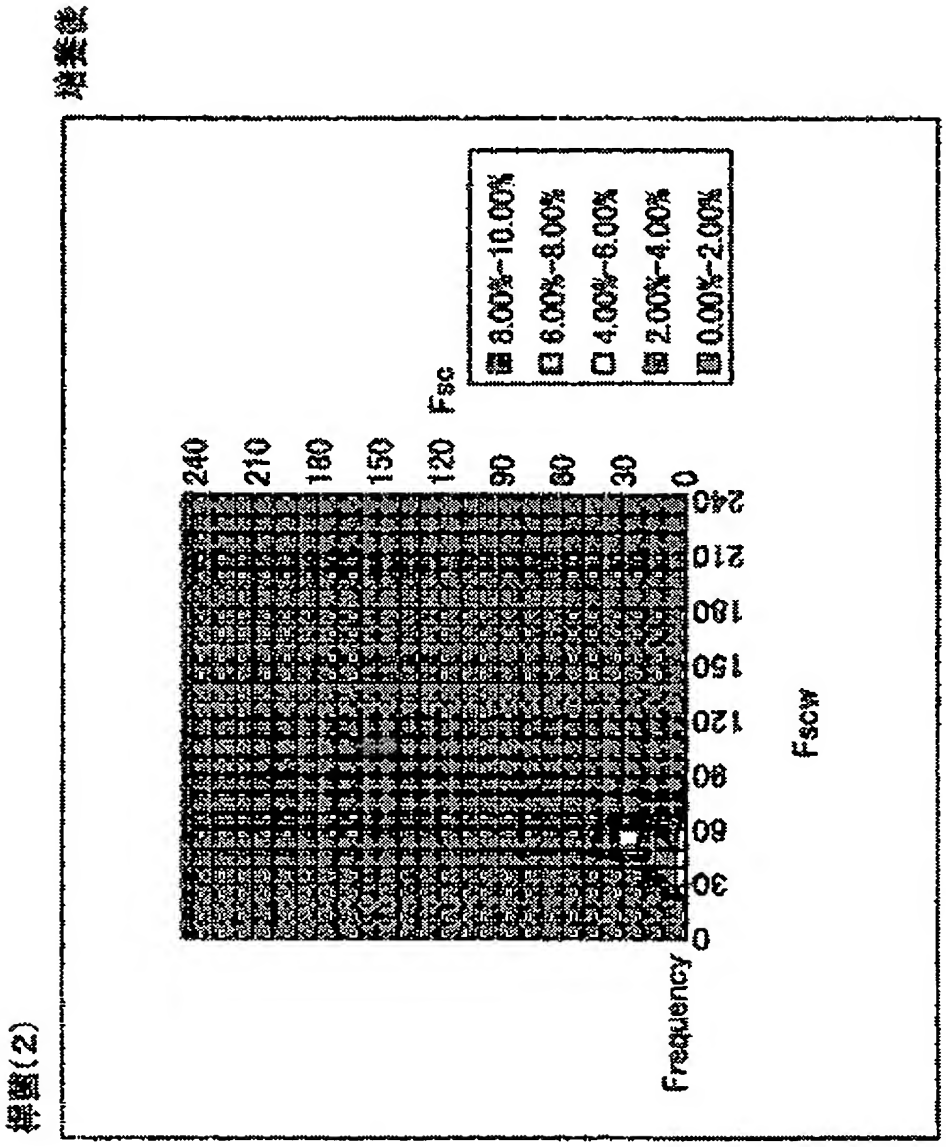
【図２４】

図面代用写真（カラー）



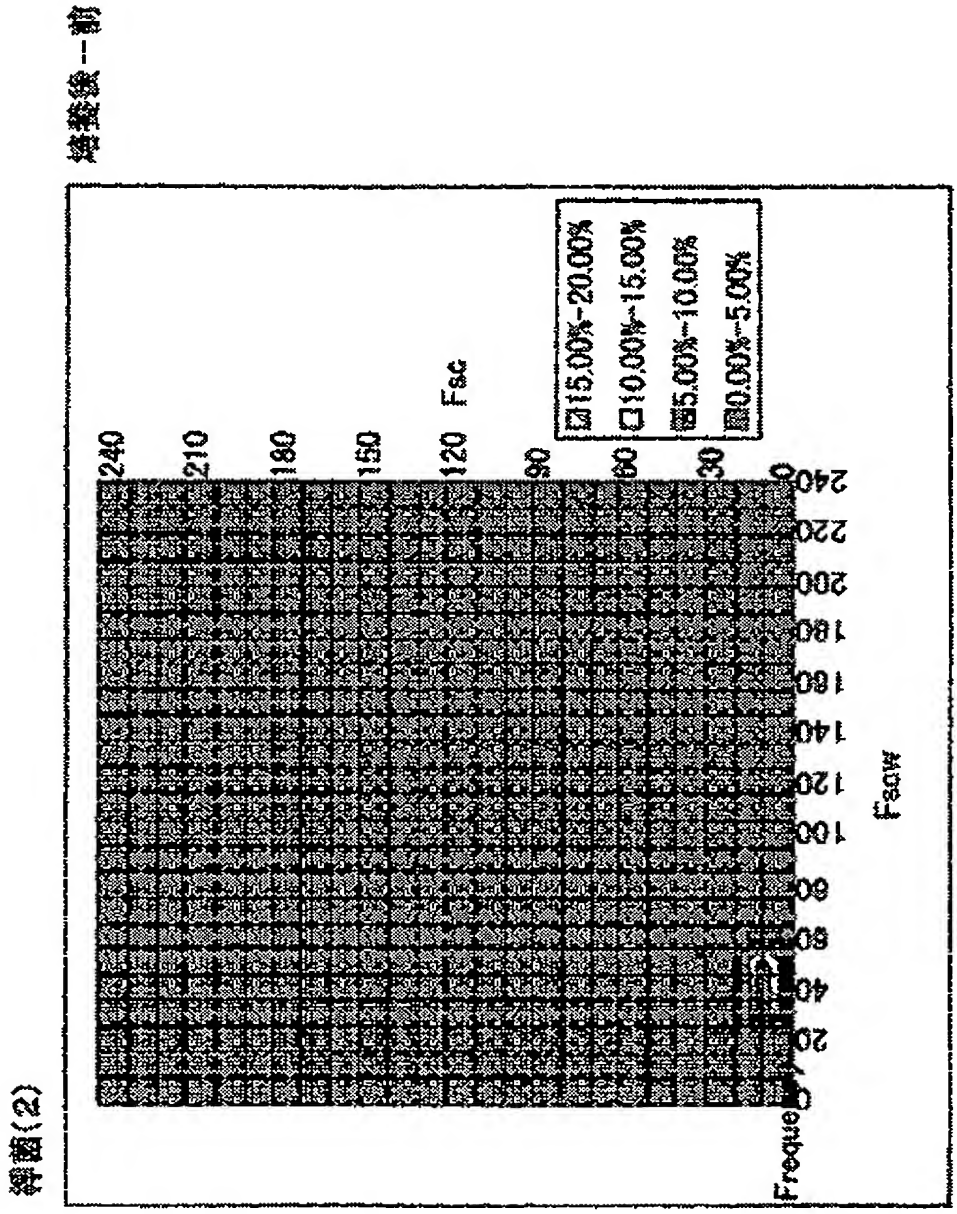
【図２５】

図面代用写真（カラー）



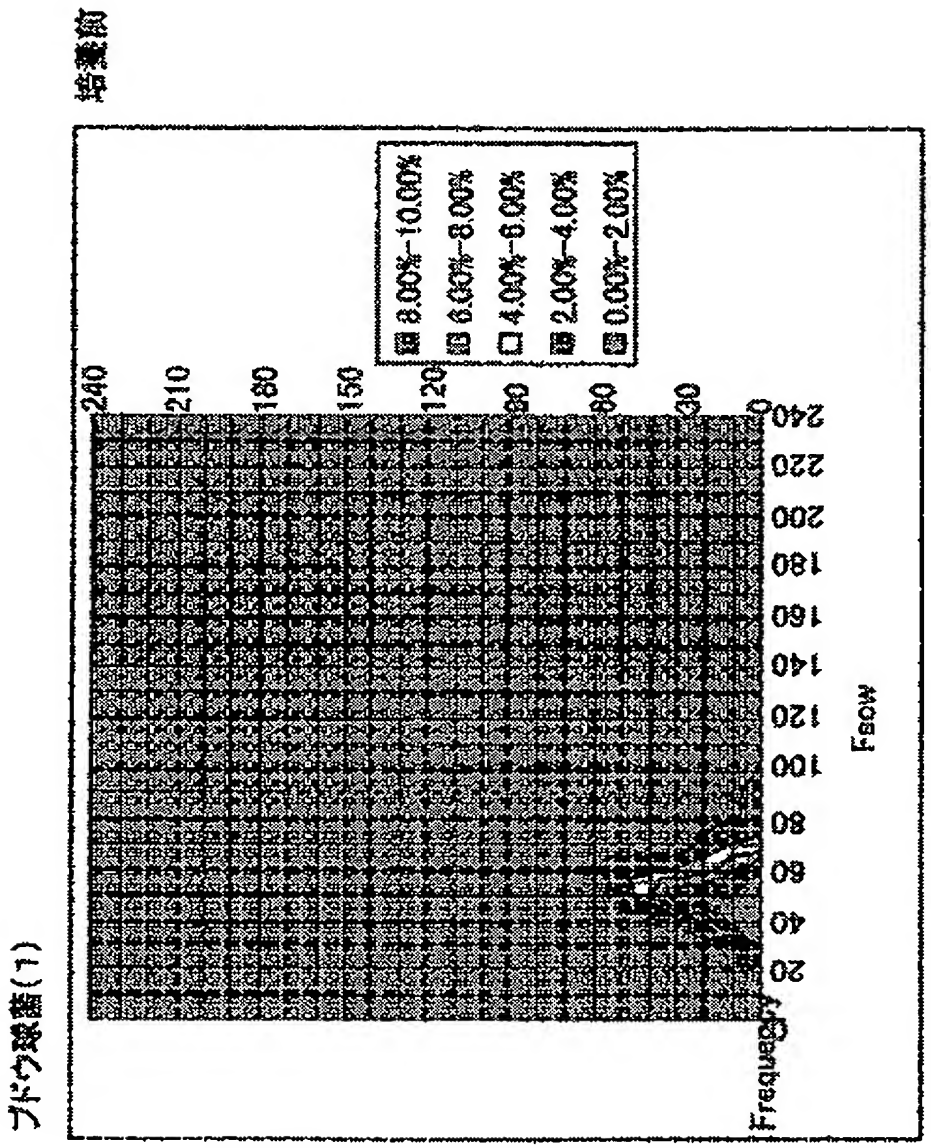
【図２６】

図面代用写真（カラー）



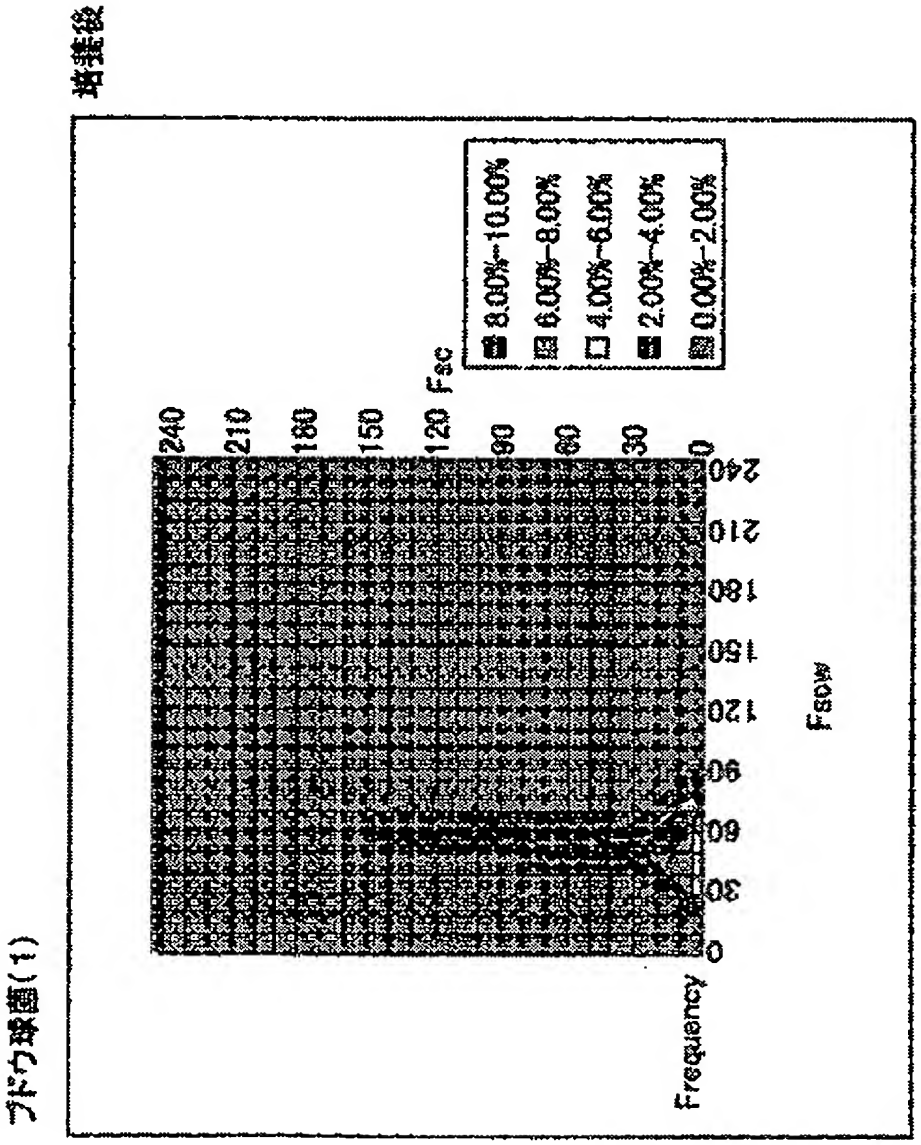
【図２７】

図面代用写真（カラー）



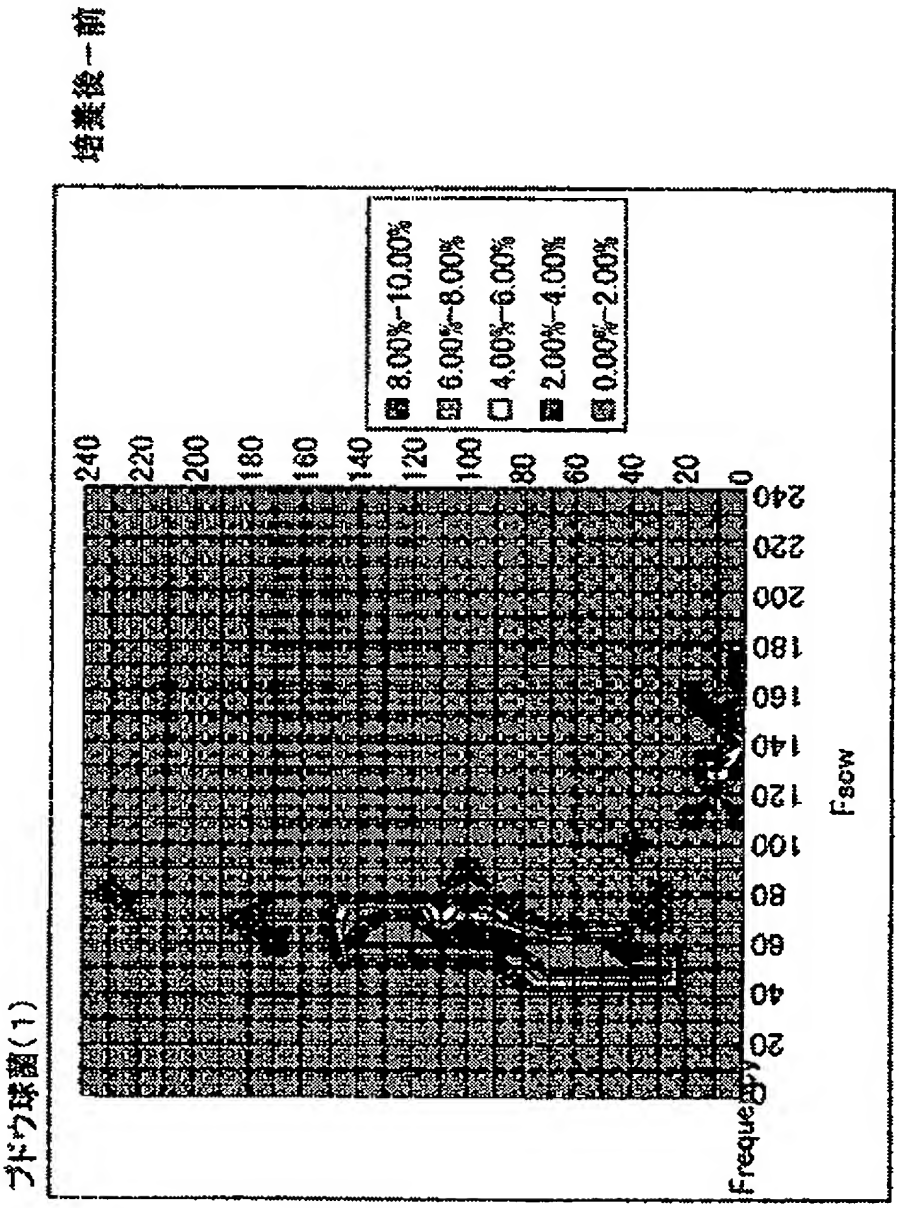
【図２８】

図面代用写真（カラー）



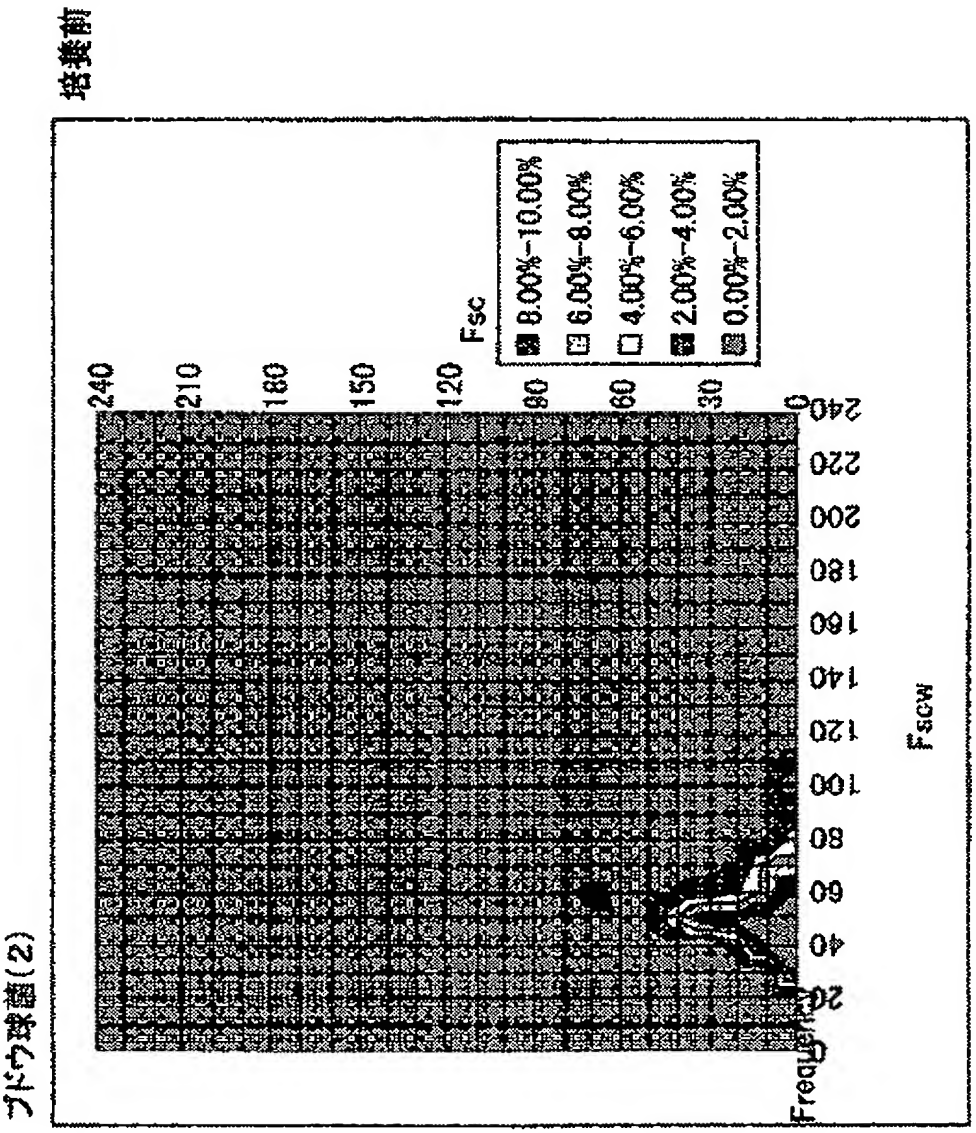
【図29】

図面代用写真（カラー）



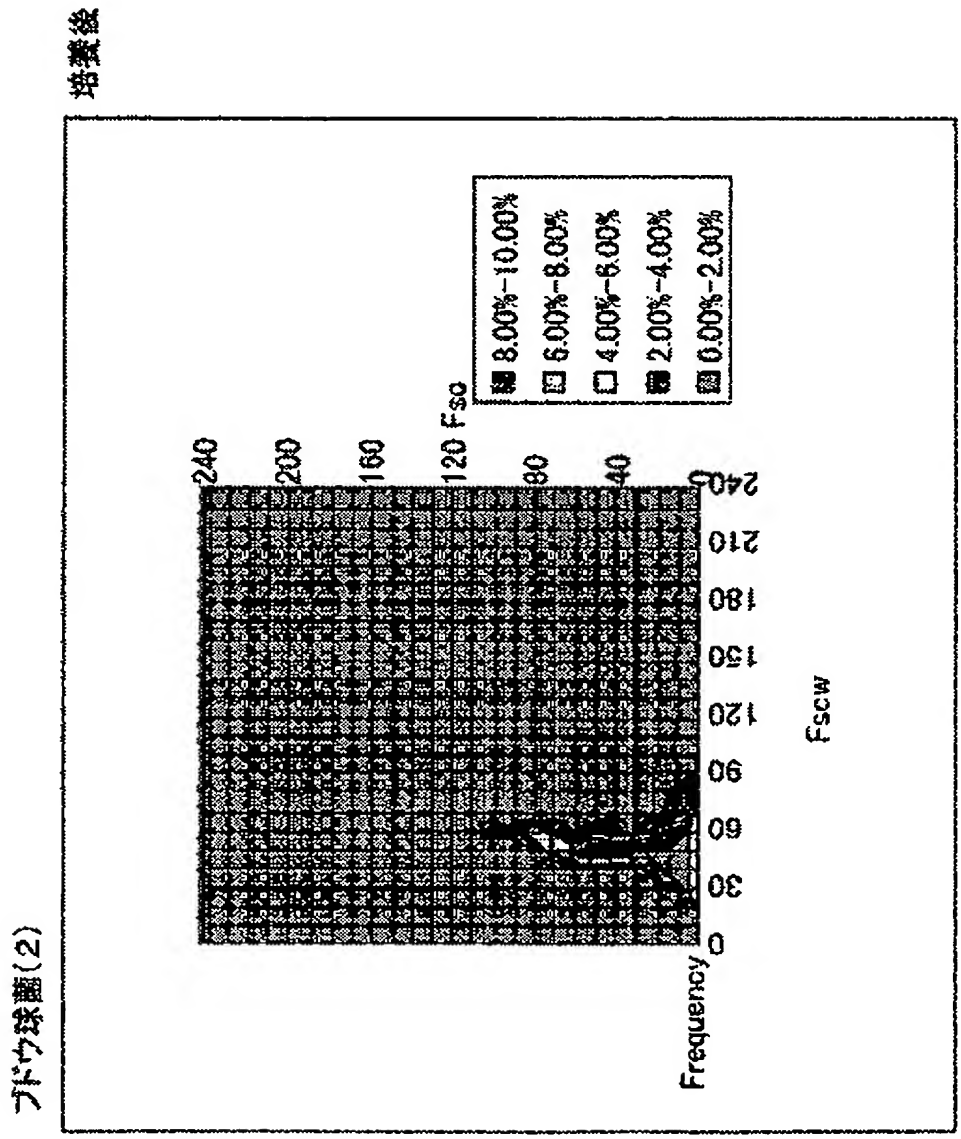
【図３０】

図面代用写真（カラー）



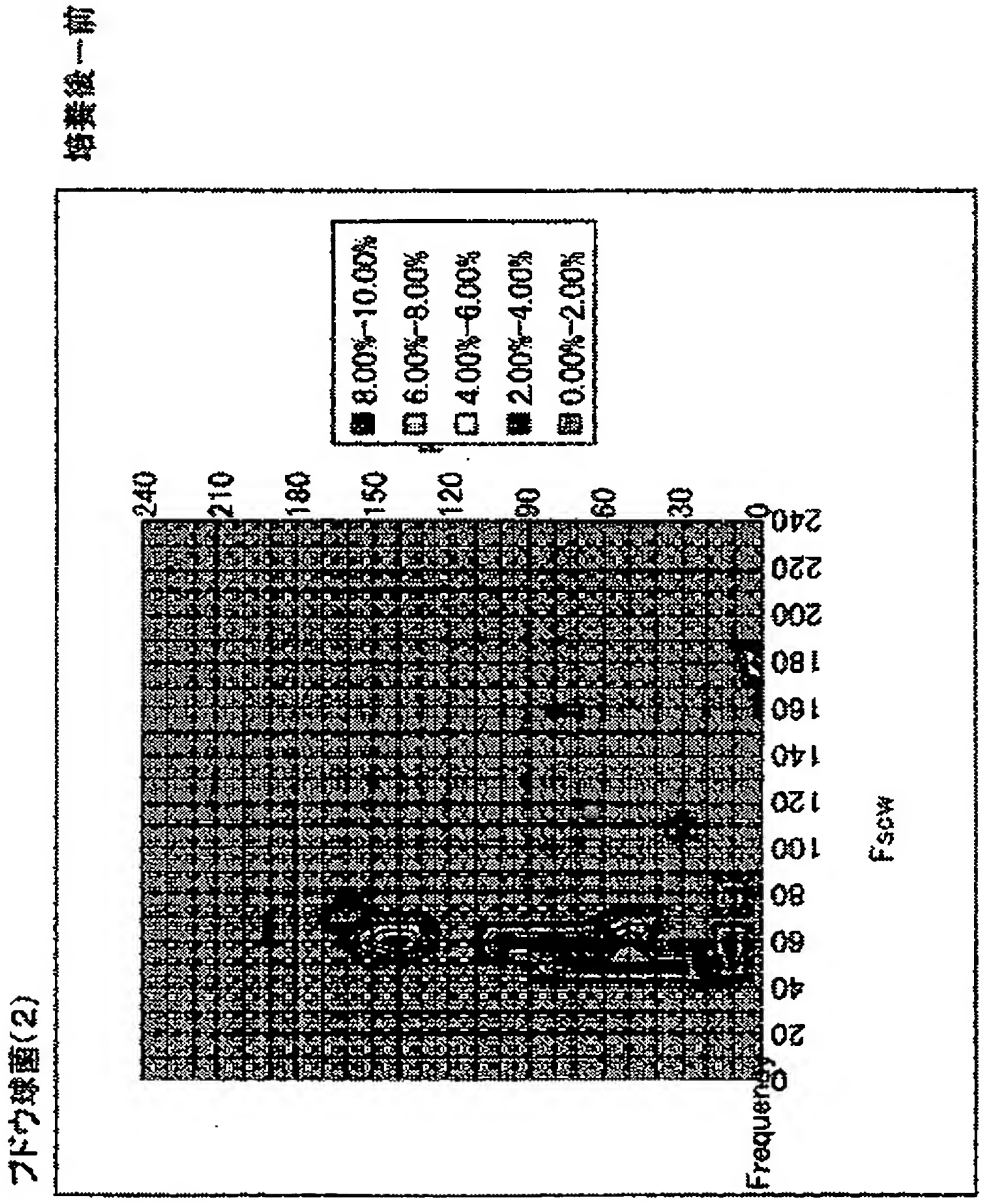
【図３１】

図面代用写真（カラー）



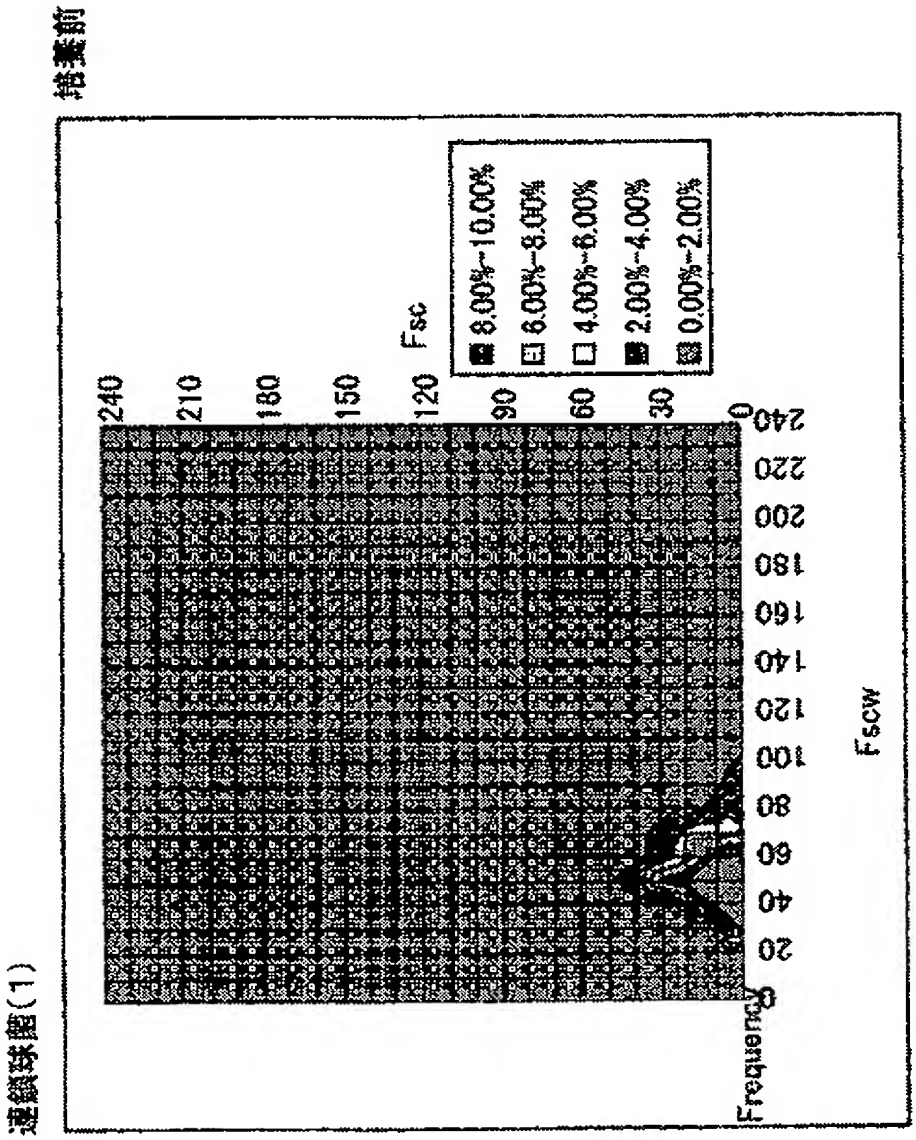
【図３２】

図面代用写真（カラー）



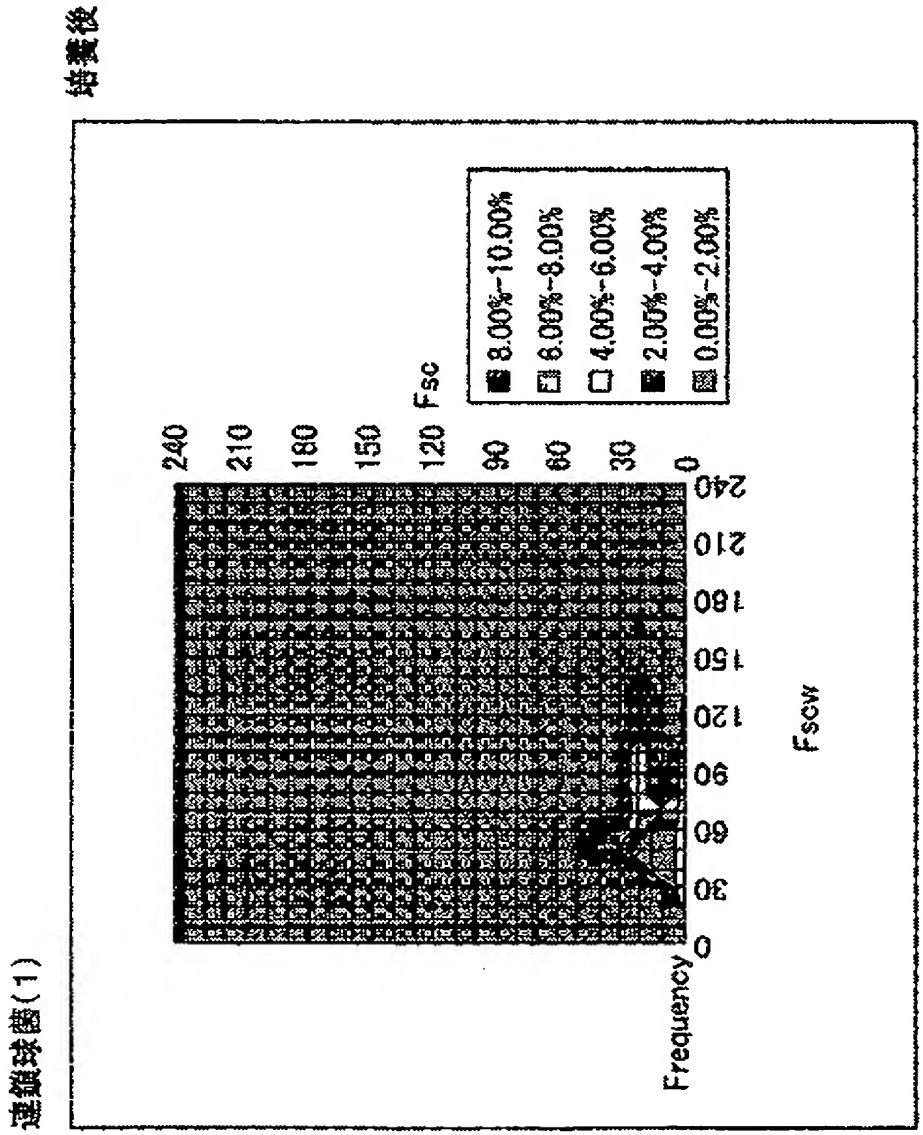
【図３３】

図面代用写真（カラー）



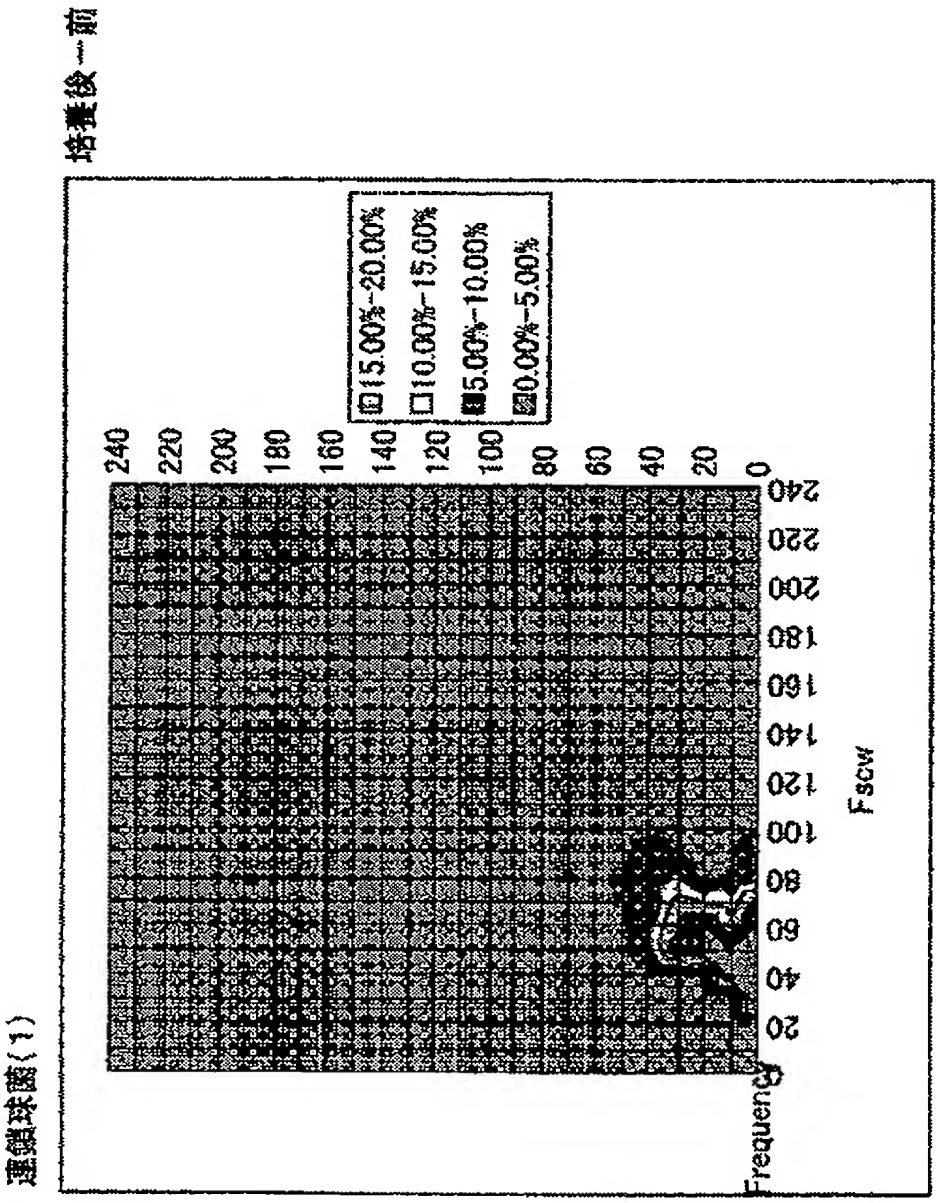
【図３４】

図面代用写真（カラー）



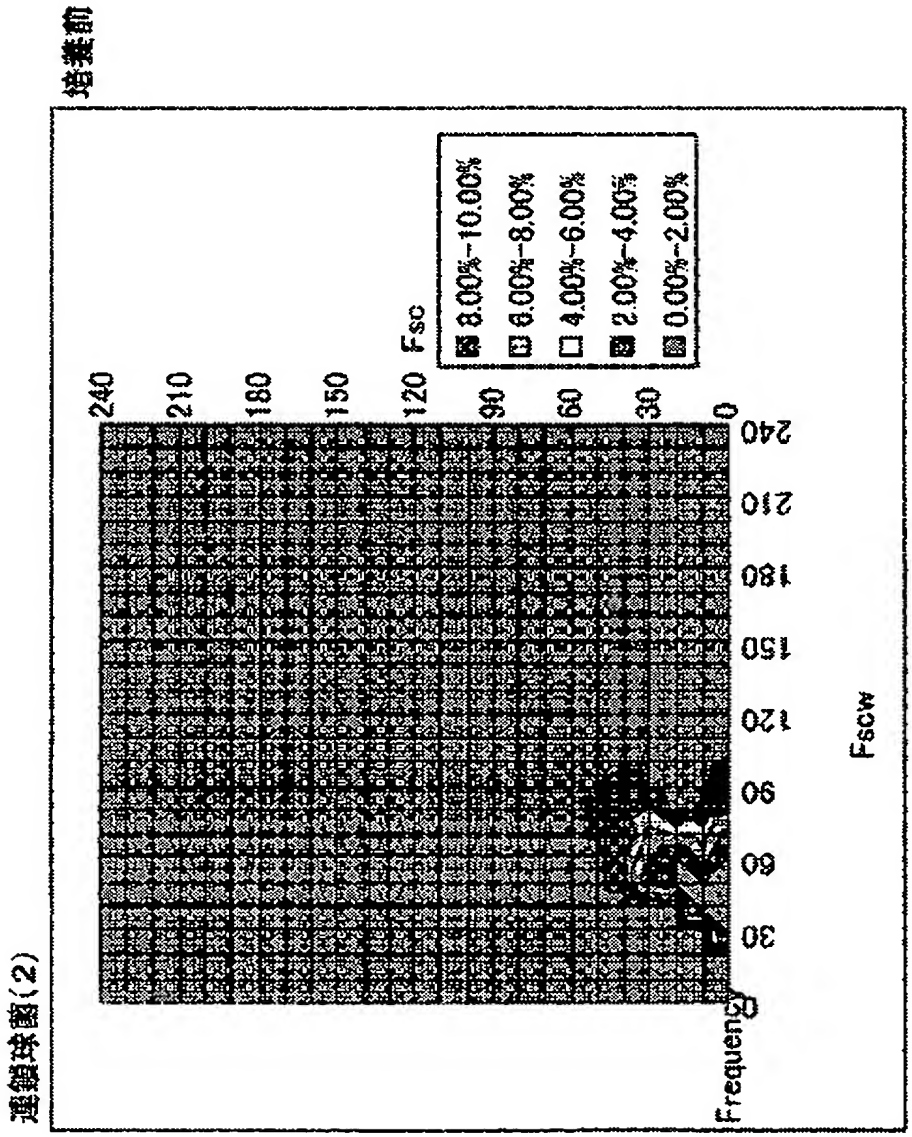
【図３５】

図面代用写真（カラー）



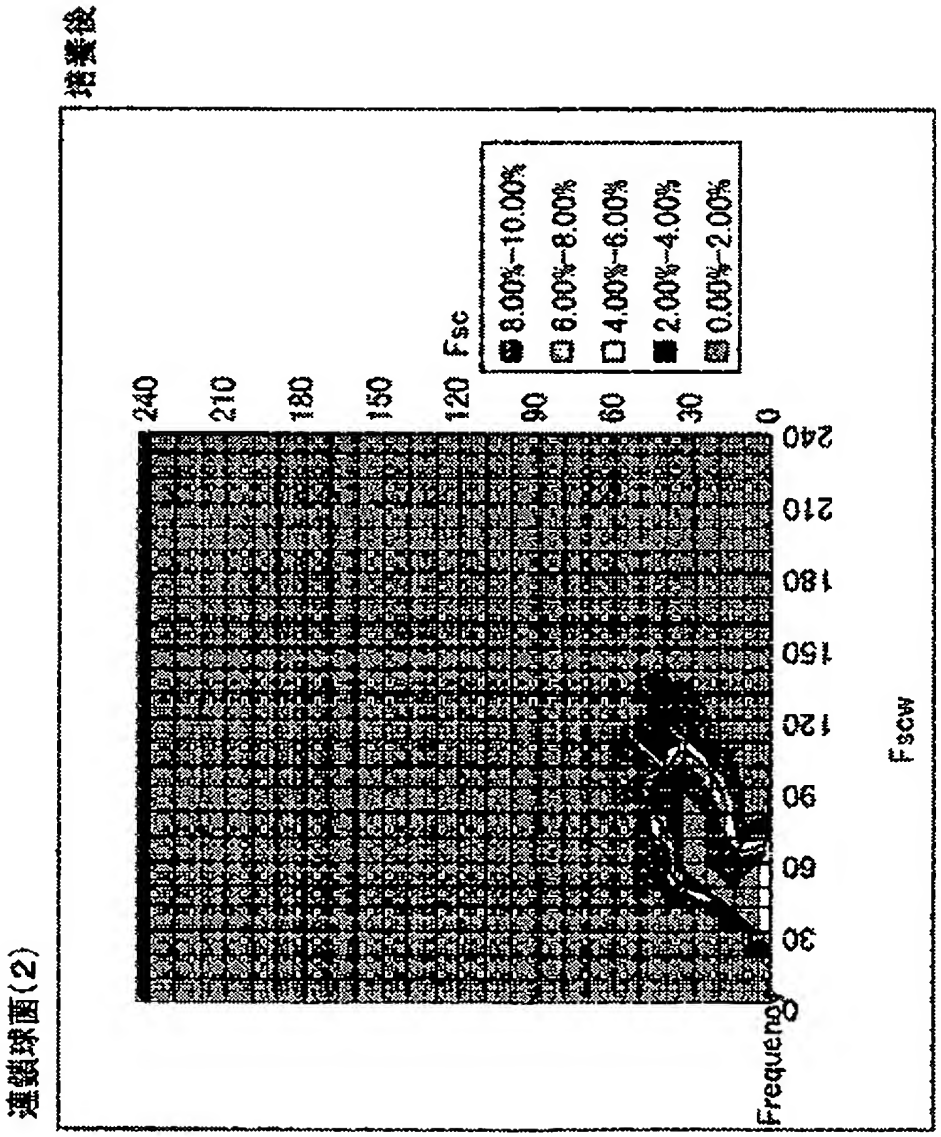
【図３６】

図面代用写真（カラー）



【図３７】

図面代用写真（カラー）



【図３８】

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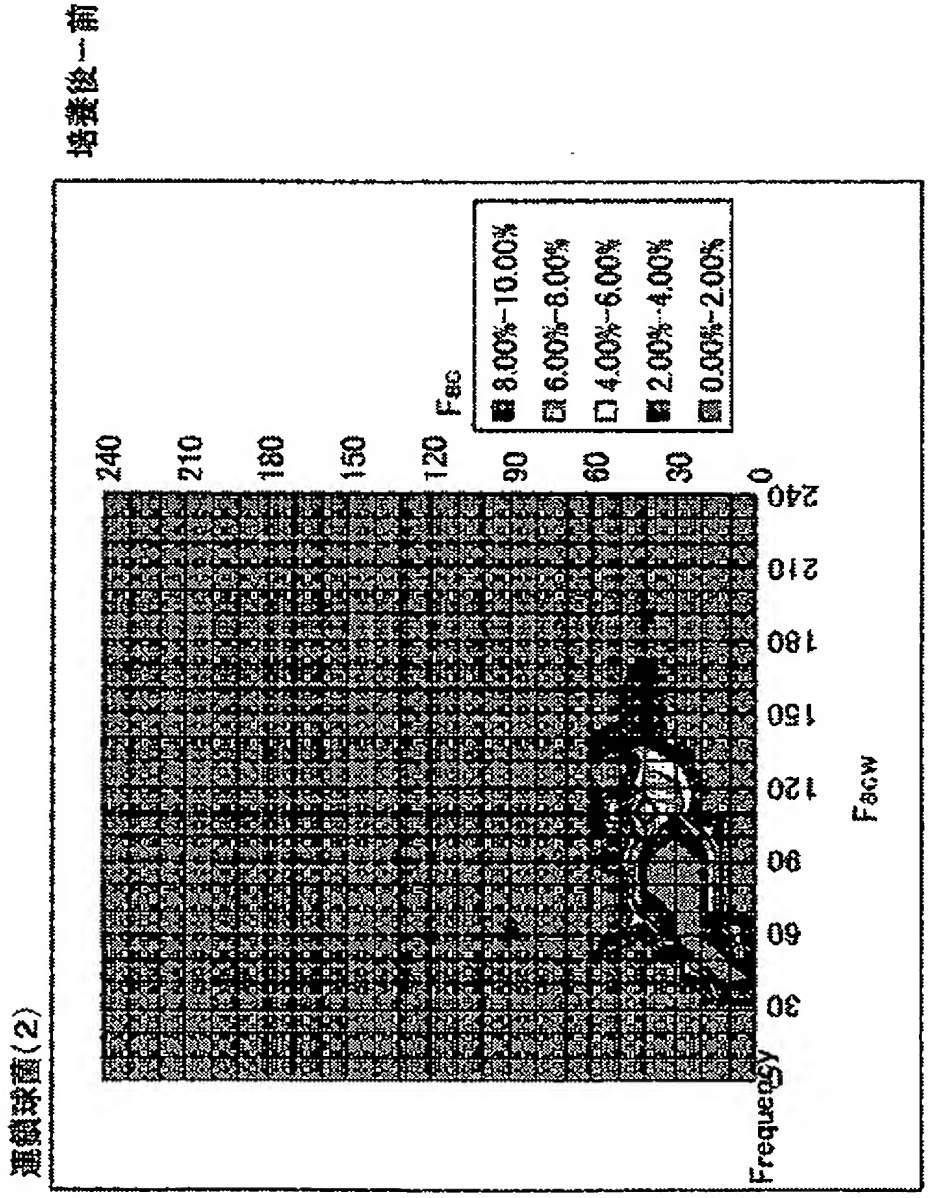

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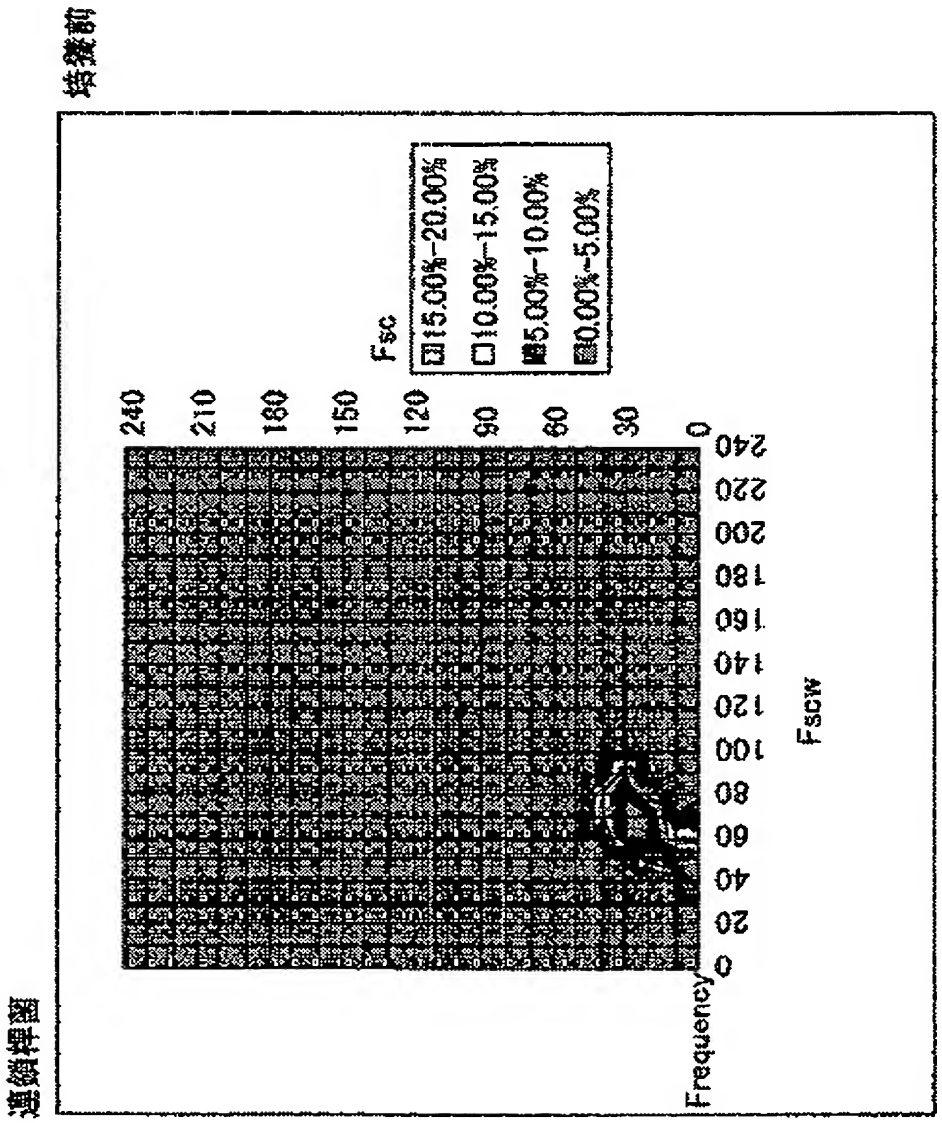

【図４０】

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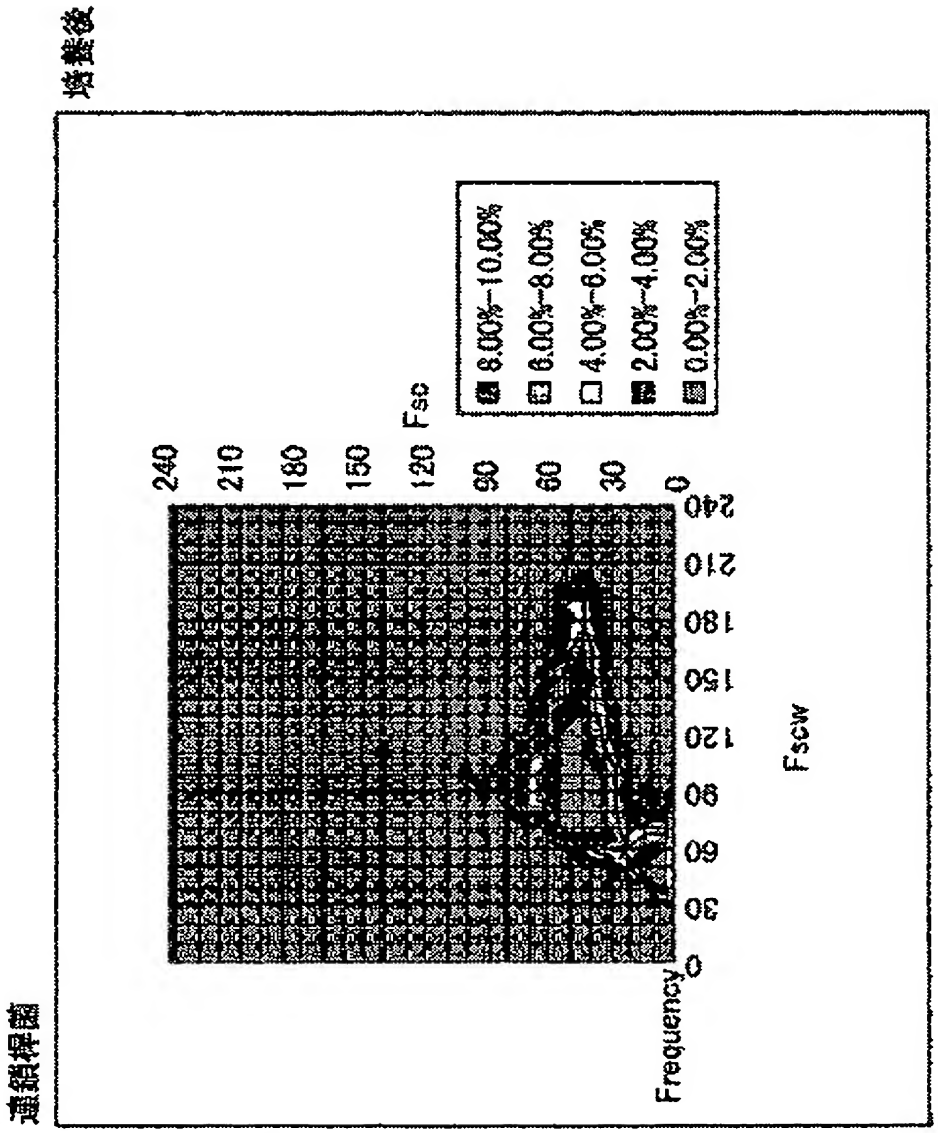

【図４１】

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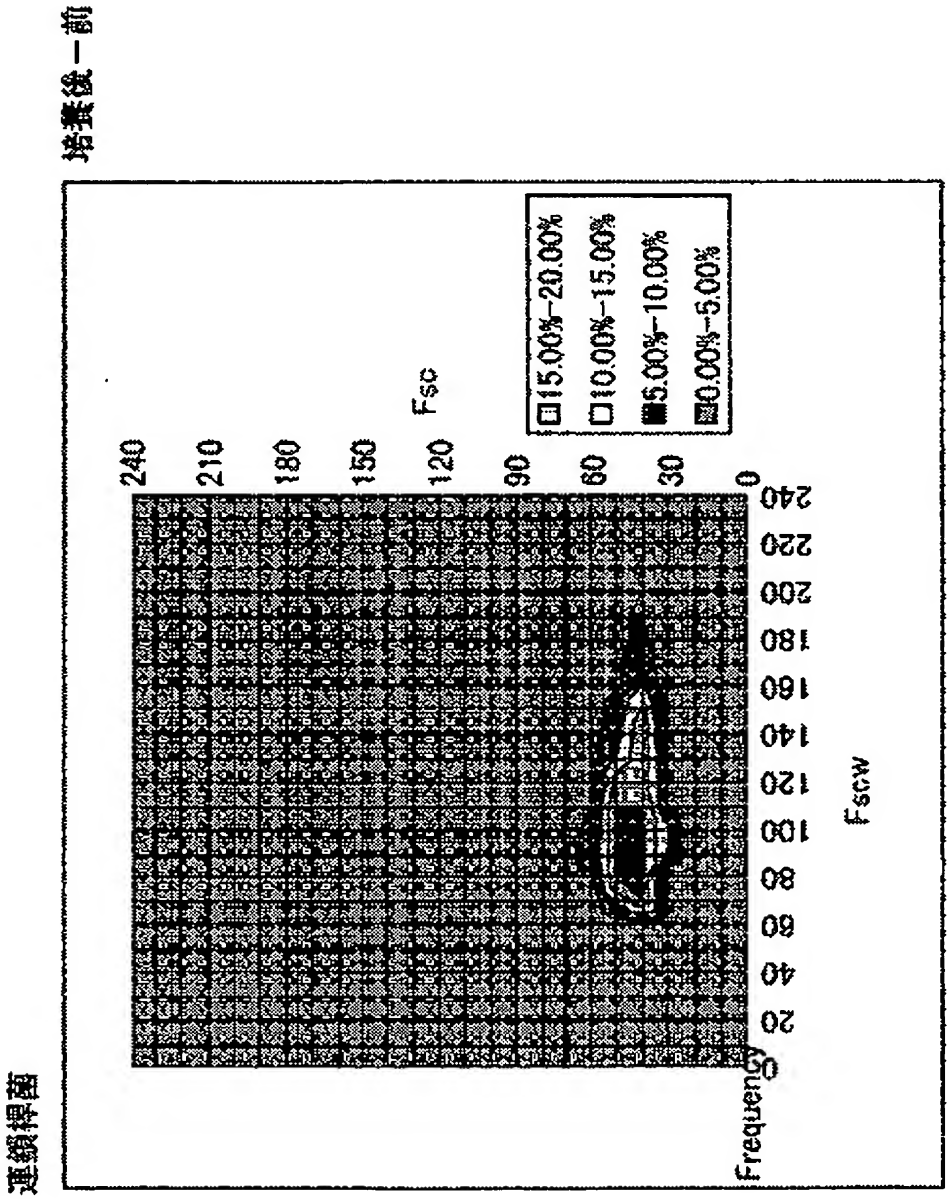

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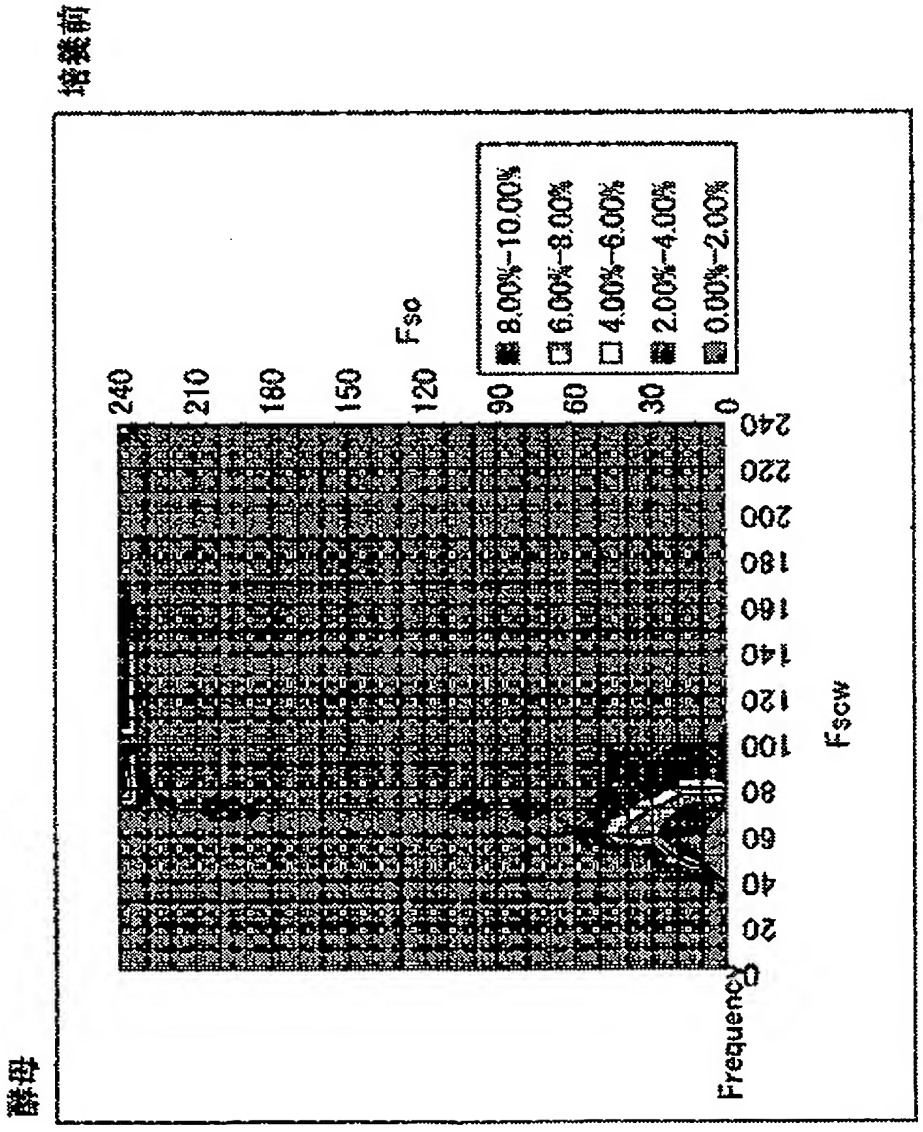

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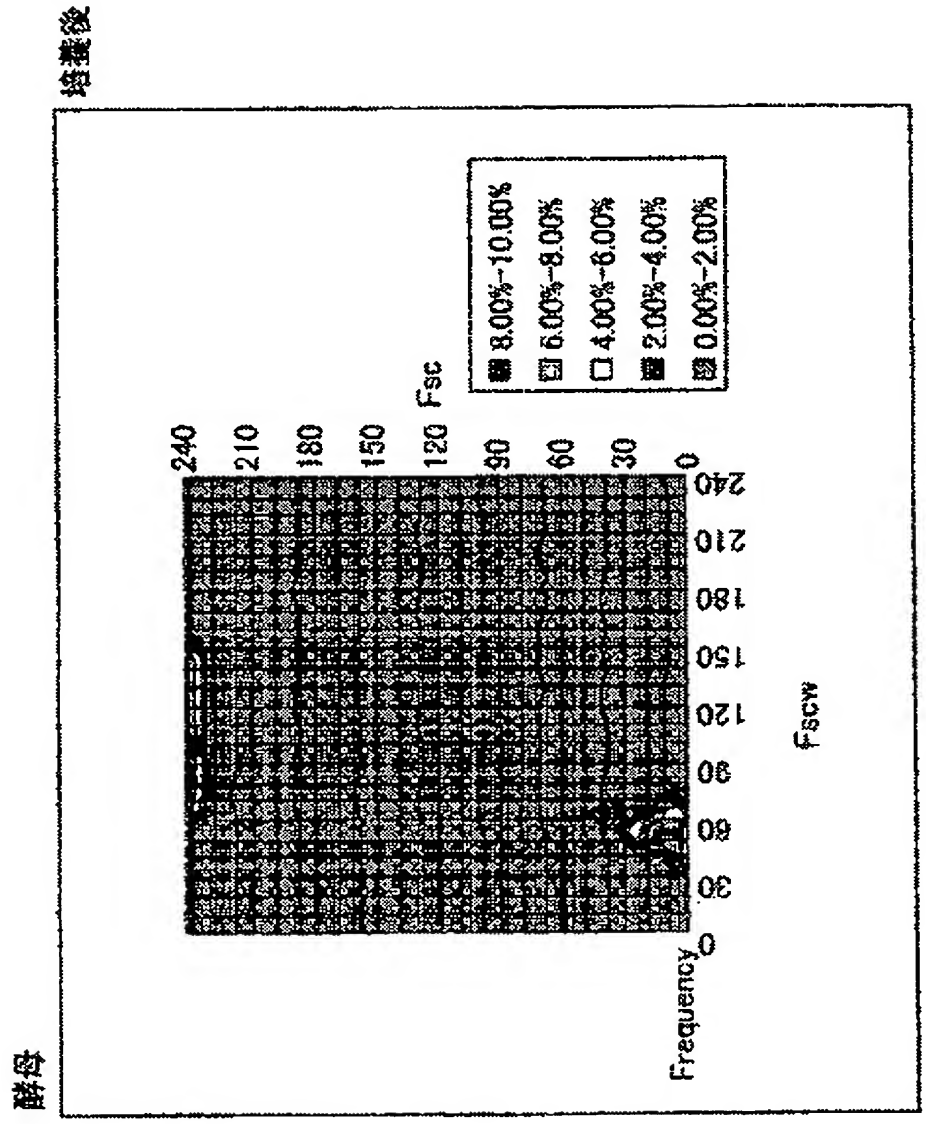

【図４４】

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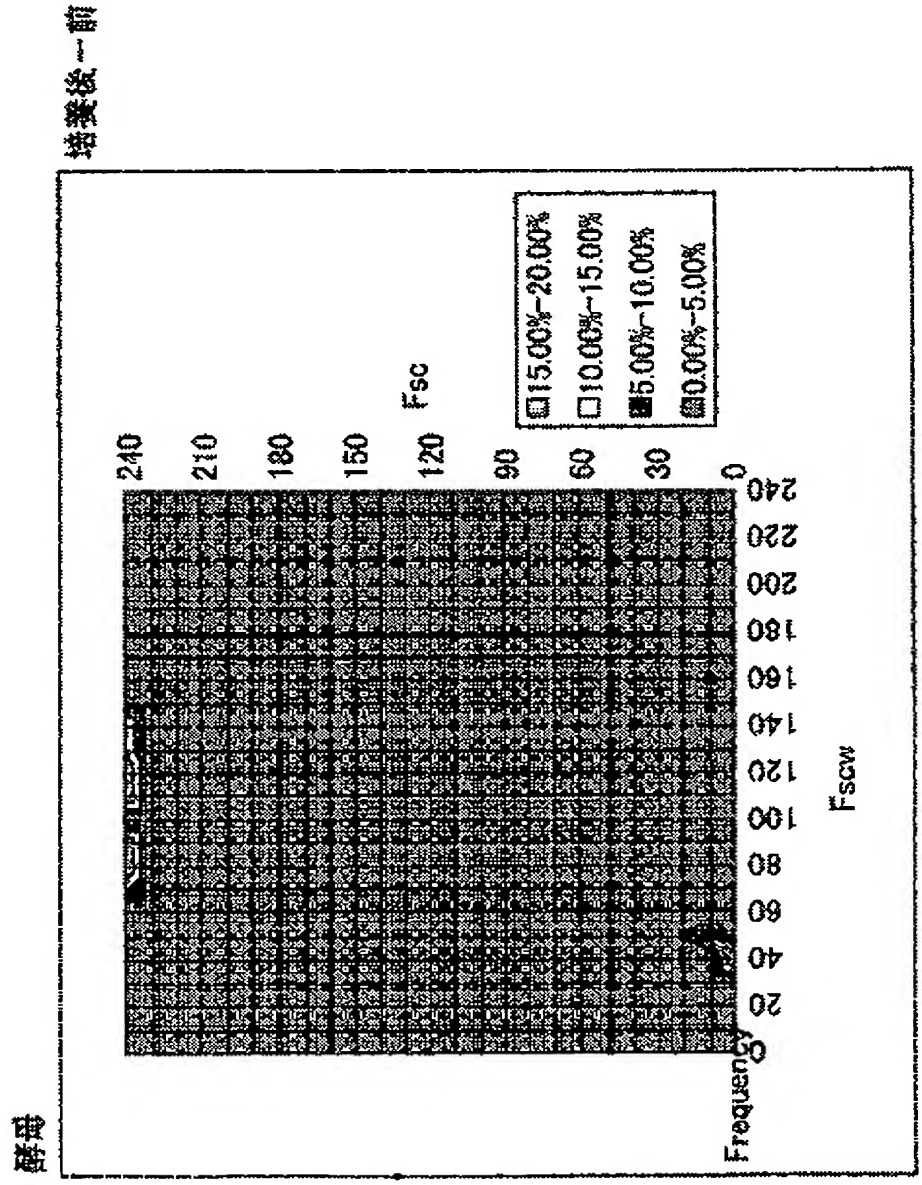

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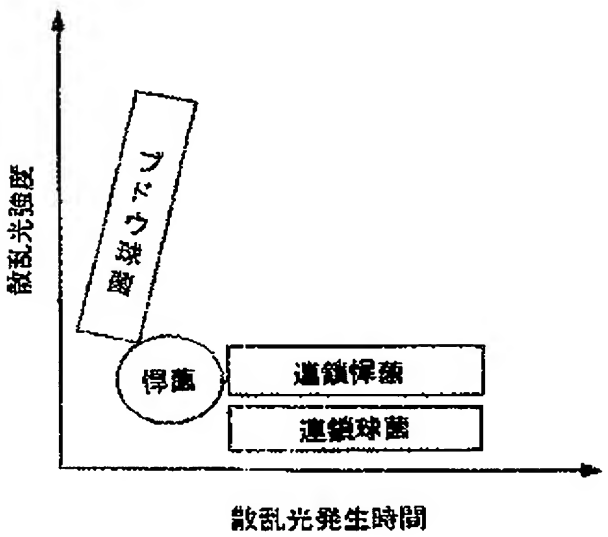
【提出日】平成１３年２月１日（２００１．２．１）
【手続補正１】
【補正対象書類名】図面
【補正対象項目名】図４５
【補正方法】変更
【補正内容】
【図４５】

面積による粒度分布の違いを示す模式図



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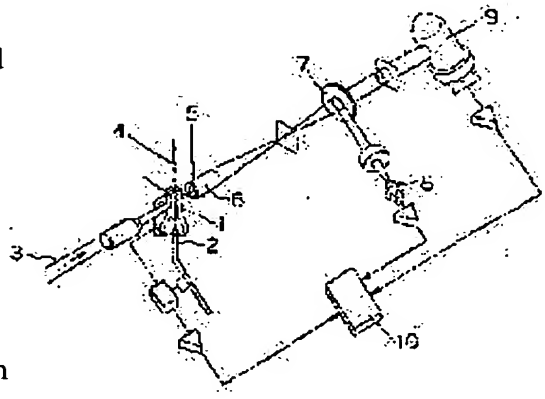
(54) METHOD FOR ASSAYING MICROORGANISM AND DEVICE THEREFOR

(57)Abstract:
PROBLEM TO BE SOLVED: To reduce the influence of impurities on the assay of microorganisms and shorten the time occupied on the assay.
SOLUTION: This assay of microorganisms is carried out before and after each cultivation to take the difference between before and after. By this process, the assay is prevented from error due to the influence of impurities contained in a sample. The microorganisms can be assayed even though the cultivation time is short, because the assay of the microorganisms is carried out by flow cytometry. Moreover, the assay is accurate, because the impurities are not measured. Further, the growth form of the microorganisms is clarified by assaying the change in the luminescent strength of forward scattering light on the luminescent time detected by flow cytometry. Accordingly, the microorganisms can be divided roughly into five categories of bacillus, staphylococcus, streptobacillus, streptococcus and yeast-like mycete from the difference of particle size distribution between before and after each cultivation.



DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention]This invention relates to the method of cultivating the microorganism in which bacteria and other culture are possible, and measuring number of microorganism. Especially, also in the sample in which impurity other than a microorganism is contained, it is related with the microorganism measuring method which measures number of microorganism to high sensitivity by short-time culture.
[0002]
[Description of the Prior Art]Conventionally, general methods of cultivating a microorganism and measuring the number of microorganisms include an agar plate smear method. In this method, the smear of a constant rate of samples is carried out on the agar plate culture medium containing a moderate nutrient, and after cultivating until a colony becomes a size observable under a naked eye or a microscope, the produced colony count is measured. However, if this method is used, it must wait for a bacillus colony to grow to the grade which becomes observable under a naked eye or a microscope. Therefore, in order to guess the kind of bacillus to some extent, the incubation period of about 18 to 24 hours is usually required. Depending on a strain, incubation period also has a thing over long periods of time, such as 24 hours or more, 48 hours or more, or January.
[0003]The drug susceptibility testing for mycobacterium which investigates the validity of the identification inspection of the strain for specifying a microorganism depending on the kind of bacillus increased by culture or drugs may be needed. However, it is difficult to take time in said method, before these necessity becomes clear, and to inspect many samples efficiently. There is a method of using a liquid medium instead of the above-mentioned agar plate culture medium, and cultivating a sample. This method cultivates by mixing a constant rate of samples with the liquid medium containing a moderate nutrient, and measures turbidity with a naked eye, an absorbance altimeter, a spectrophotometer, etc. However, since sensitivity says a photometer but there is, it must wait for growth of a bacillus till the grade from which a nephelometry changes also in this method. [no] Therefore, like an agar plate smear method, the incubation period for how many days is required for measurement of number of microorganism, and there is the above-mentioned problem in it.
[0004]The sample under culture is irradiated and the method of measuring number of microorganism from the change to the time of the scattered light and the transmitted light is indicated by JP,5-82901,A. Even if this method is a bacillus in which growth gestalten differ, it can search for number of microorganism correctly. However, in order to use the change of scattered-light data and the change of transmitted light data in the growth course of a bacillus for this method, the time which culture of a sample takes is not shortened.
[0005]
[Problem(s) to be Solved by the Invention]Particle measuring devices, such as flow

cytometer, are known as a device which, on the other hand, measures the particles of a minute size like a microorganism to high sensitivity. Since a particle measuring device measures the number of the particles in a sample one by one, high sensitivity measurement is possible for it. Therefore, if a particle measuring device is used, even if it does not carry out long term culture, the bacterial count in a sample is measurable.

[0006]However, a particle measuring device will detect impurity, such as the particles of size about the same as a microorganism, for example, dust, and a sludge, as a microorganism. For this reason, the error by impurity will be included in the detection result of a particle measuring device. For example, when measuring bacteria by making urine into a sample, red corpuscles, leucocytes, epithelial cells, a pillar, a crystal, etc. which are the physical components in the urine, and its collapse thing will be detected as bacteria.

[0007]Then, in order to identify impurity and a microorganism, a microorganism is dyed and the method of measuring the fluorescence shown from a microorganism is proposed. However, a dyeing degree changes with kinds of microorganism. Fluorescent dye and the processing condition which dye only the target microorganism must be set up for every sample. For this reason, measurement takes time and effort and it is unsuitable for analyzing a lot of samples.

[0008]Otherwise, change of the impedance of the culture medium accompanying growth of a microorganism, change of PH of culture medium, the amount of consumption oxygen, or the amount of generating carbon dioxide is measured by making a microbial count into the method of detecting promptly, and the method of asking for a microbial count from correlation of these and a microbial count is studied these days. However, said measured value may change with causes other than the cultivated microorganism. Since the method of satisfying from the detection limit of a microorganism and a point of detecting accuracy cannot say, it is the method of using only by the specific condition lower. That is, the method of judging is not yet provided with the microorganism in a sample for a short time and and correctly as mentioned above.

[0009]This invention is a thing in consideration of the above situations, and an object of this invention is to provide the method of measuring measurement of a microorganism with simply sufficient accuracy by short-time culture.

[0010]

[Means for Solving the Problem]In order that the 1st invention of this application may solve the above-mentioned technical problem, it is a measuring method of a microorganism in a culture sample which added a sample of a measuring object to culture medium, The 1st measuring process that measures said culture sample after carrying out A; predetermined time culture processing using said flow cytometer, and acquires the 1st particle size distribution of said culture sample from optical information of the scattered light detected, B; The 2nd measuring process that measures a culture sample before said culture processing using said flow cytometer, and acquires the 2nd particle size distribution of said culture sample from optical information of the scattered light detected, An analysis process which acquires particle size distribution of a microorganism cultivated in said culture sample from difference of the 1st particle size distribution of C; above, and the 2nd particle size distribution, and D; a microorganism measuring method including an output process which outputs particle size distribution of said obtained microorganism is provided.

[0011]By subtracting a measurement result before culture from a measurement result after culture, it can prevent measuring as a microorganism impurity contained in a sample. Impurity is a physical component in dust or a sample, for example. In said 1st invention, the 2nd invention of this application measures emission time and intensity of a scattered-light signal which are detected using said flow cytometer, and provides a microorganism measuring method acquiring said 1st and 2nd particle size distribution.

[0012]Scattered light intensity gives information about a size of grain contained in a culture sample. Scattered-light emission time gives information about the length of grain contained in a culture sample. Therefore, vegetative form voice of increasing to a chain or increasing to tufted [of a grape] can be known by measuring change of intensity to emission time. The 3rd invention of this application by measuring emission time and intensity of a scattered-light signal which are detected using said flow cytometer in said 1st invention, Particle size distribution of said cultivated microorganism is acquired, vegetative form voice of said microorganism is presumed based on said particle size distribution, and a microorganism measuring method judging to any of a predetermined classification said microorganism belongs is provided.
[0013]When a bacillus whose length whose path is comparatively large and is not so long is increasing, Staphylococcus, Streptococci can be presumed when a bacillus with them is increasing. [streptobacillus, a path still smaller than streptobacillus, and] [long when a bacillus with them is increasing] [a Bacillus, a comparatively small path, and] [long when a bacillus with short length whose path is comparatively small is increasing] The 4th invention of this application acquires particle size distribution of said cultivated microorganism by measuring emission time and intensity of a scattered-light signal which are detected using said flow cytometer in said 1st invention, A microorganism measuring method judging to any vegetative form voice of said microorganism shall belong between Staphylococcus, streptococci, streptobacillus, or a Bacillus based on said particle size distribution is provided.
[0014]It has the same operation effect as said 3rd invention. The 5th invention of this application provides a microorganism measuring method, wherein a measuring object sample is urine in said 1st invention. When making urine into a sample, a classification result of said microorganism to Gram negative / positive judgment is possible.
[0015]The 6th invention of this application provides a microorganism measuring method characterized by measuring bacteria and/or yeast-like fungi as a microorganism in said 1st invention. The 7th invention of this application is used with flow cytometer which measures a particle size of a microorganism in a culture sample which added a measuring object sample to culture medium, and provides a microorganism measuring device provided with the 1st measuring means, the 2nd measuring means, an analysis means, and an output means. The 1st measuring means acquires the 1st particle size distribution of a culture sample after performing predetermined time culture processing from optical information of the scattered light detected by said flow cytometer. The 2nd measuring means acquires the 2nd particle size distribution of a culture sample before said culture processing from optical information of the scattered light detected by said flow cytometer. An analysis means acquires particle size distribution of a microorganism cultivated in said culture sample from difference of said 1st particle size distribution and the 2nd particle size distribution. An output means outputs particle size distribution of said obtained microorganism.
[0016]It has the same operation effect as said 1st invention. The 8th invention of this application is used with flow cytometer which measures a particle size of a microorganism in a culture sample which added a measuring object sample to culture medium, It is the recording medium which recorded an analysis program which analyzes a measurement result of said particle size and in which computer reading is possible, and a recording medium which recorded an analysis program for performing the following A - D stage and in which computer reading is possible is provided.
A; from optical information of the scattered light detected by said flow cytometer. A stage of acquiring the 1st particle size distribution of a culture sample after performing predetermined time culture processing, B; from optical information of the scattered light detected by said flow cytometer. A stage, D which acquire particle size distribution of a microorganism cultivated in said culture sample based on difference of a stage, the 1st particle size

distribution of C; above, and the 2nd particle size distribution which acquire the 2nd particle size distribution of a culture sample before said culture processing; a stage which outputs particle size distribution of said obtained microorganism.
[0017]It has the same operation effect as said 1st invention.
[0018]
[Embodiment of the Invention]Hereafter, this invention is explained concretely, giving the example of an embodiment. In this invention, by wrapping and passing a sample with sheath liquid, flow cytometer makes the thin sample flow by the hydrodynamics effect form, passes one particle in b sample at a time to a primary detecting element, and means the device which detects the scattered light which irradiates with c light and is emitted from particles, and fluorescence. As a light source, a semiconductor laser, argon laser, etc. can be used suitably.
[0019]The culture medium refers to the liquid medium containing the moderate nutrient for cultivating a microorganism. If a microorganism kind is not specified, the culture medium which was suitable for the kind in the culture medium for bacterium when the microorganism kind was specified can be used selectively. Culture processing is processing for cultivating a microorganism, a culture sample is put into the humidistat of a predetermined temperature (for example, 30-37 **), and the processing which promotes culture of a microorganism is said.
[0020]It is measuring the microorganism in the culture sample before culture processing is performed in the culture sample before culture processing as for measurement. Measuring, after saving a culture sample at low temperature which a microorganism does not increase also contains.
Below the <example of a 1st embodiment> explains this invention in full detail based on the example of an embodiment shown in a drawing. This invention is not limited by this.
[0021][Device] Drawing 1 is a lineblock diagram containing the primary detecting element of the flow cytometer of this invention, and a signal processor. The primary detecting element of flow cytometer has the sheath flow cell 1, the sample nozzle 2, the beam stopper 5, the collector lens 6, the dichroic filter 7, the photo-diode 8, and the photograph mull 9.
[0022]The sheath flow cell 1 forms the sample flow 4 which wrapped the sample which flows from the sample nozzle 2 in sheath liquid, and was wrapped in sheath liquid. The particles 4 of sample flow can irradiate with the laser beam 3 from the laser source which is not illustrated. The beam stopper 5 intercepts the light which penetrates the sample flow 4 directly. The collector lens 6 condenses the forward scattering light and the front fluorescence which the particles 4 emit. The dichroic filter 7 reflects forward scattering light. The photo-diode 8 detects the forward scattering light reflected by the dichroic filter 7. The detected forward scattering light is inputted into the signal processor 10 via amplifier. The photograph mull 9 detects the front fluorescence which passed the dichroic filter 7. The forward scattering light and the front fluorescence which were detected by the photo-diode 8 and the photograph mull 9 are inputted into the signal processor 10 via amplifier, respectively.
[0023]The signal processor 10 concerning this invention receives the detecting signal outputted from the photo-diode 8, Change of the scattered light intensity (Fsc;Forwardscatter intensity) to the emission time (Fscw;Forward scatter pulse width) of forward scattering light is measured. The signal processor 10 creates and analyzes size distribution figures based on a forward scattering lightwave signal. The key map of a signal inputted into the signal processor 10 is shown in drawing 2. Scattered light intensity is equivalent to the intensity, i.e., the pulse height, of an input signal. The emission time of the scattered light is equivalent to the pulse width of an input signal.
[0024]Drawing 3 is a block diagram showing the functional constitution of the signal processor 10. The signal processor 10 is formed, for example in information terminals, such as PC (Personal Computer) and WS (WorkStation), and outputs a processing result to the

outputting part of an information terminal. The signal processor 10 is provided with the particle-size-distribution preparing part 101, the storage parts store 102, the treating part 103, and the output control part 104.
[0025]The particle-size-distribution preparing part 101 receives the lightwave signal which flow cytometer detected, and creates the particle size distribution which makes a horizontal axis forward scattering photoluminescence time, and makes a vertical axis forward scattering photoluminescence intensity based on an input signal. An output is possible for the particle-size-distribution preparing part 101 to outputting parts, such as a display, via the output control part 104 in the size distribution figures showing the created particle size distribution. Drawing 6 mentioned later, drawing 7, etc. are examples of the size distribution figures outputted by the particle-size-distribution preparing part 101.
[0026]The storage parts store 102 memorizes the created size distribution figures about each of after culture and a front. The storage parts store 102 memorizes the analysis result of division data and size distribution figures which are created based on size distribution figures. Division data and an analysis result are mentioned later. The treating part 103 subdivides size distribution figures to a predetermined division, and asks for the data number (only henceforth division data) in each division. In other words, division data is a particle number contained in one division on size distribution figures. The treating part 103 asks for a changed part of the division data in the same division in the particle size distribution of the back before culture. Thereby, the error of measurement by measuring the impurity in a sample as a microorganism can be decreased.
[0027]The error of measurement by impurity is explained. 1 micrometer or less of impurity of size about the same as the microorganism in culture medium also detects the detection by the forward scattering light intensity of flow cytometer. Many particles are detected also before culture processing (refer to drawing 6 mentioned later). Even if many particles are detected after culture processing, it cannot be judged whether it is the impurity which is mixing from the beginning whether it is the microorganism which it increased. Then, the particle size distribution before culture processing is subtracted from the particle size distribution after culture processing, and the particles which increased by culture processing, i.e., the cultivated microorganism, are detected.
[0028]Based on a part for each division data or the change, the treating part 103 analyzes particle size distribution by a predetermined method, and outputs an analysis result to a display control part. For example, when a division with most changed parts is made into 100%, the changed rate of each division data is searched for, and using the division below a predetermined rate as a display portion is mentioned. The treating part 103 sets the color according to the degree of change as each division, in order to display the degree of change of division data visually. The treating part 103 may set the color according to the size of division data as each division similarly about the particle size distribution of the back before culture.
[0029]The output control part 104 outputs each division to outputting parts, such as a display and a printer, using the foreground color set up by the treating part 103. Drawing 21 mentioned later - 28 are the display examples of the analysis result outputted by the output control part 104. About scattered light intensity, the horizontal axis of a vertical axis is the emission time of the scattered light. For example, drawing 21 shows about a Bacillus the analysis result of the particle size distribution of the microorganism which increased by the particle size distribution before culture, the particle size distribution after culture, and culture, respectively.
[0030][Flow of processing] Next, the flow of the analysis processing which the signal processor 10 performs is explained. Drawing 4 is a flow chart which shows an example of the flow of the analysis processing which the signal processor 10 performs. The following

processings are started by inputting a detecting signal from flow cytometer. First, in Step S1, the treating part 103 performs predetermined initialization processing. Specifically, the treating part 103 is set to n= 1, x= 1, and y= 1. Here, n is a variable which shows a measurement count. x and y are variables which show the subdivided position of x shaft orientations of the division of size distribution figures, and y shaft orientations.

[0031]In Step S2, the particle-size-distribution preparing part 101 receives a detecting signal from flow cytometer. In Step S3, the particle-size-distribution preparing part 101 asks for the luminescence intensity to the emission time of forward scattering light based on the received detecting signal, and creates particle size distribution. Furthermore, the particle-size-distribution preparing part 101 stores said created particle size distribution in the storage parts store 102. The particle-size-distribution preparing part 101 reads particle size distribution from the storage parts store 102 automatically, corresponding to the directions from a user, and an output is possible for it on a display etc.

[0032]In step S4, the treating part 103 subdivides size distribution figures in the predetermined number of divisions. For example, it subdivides to the division (1<=x<=256, 1<=y<=256) of 256x256. In Step S5, the treating part 103 asks for the division data for every division about all the divisions, and stores it in a storage parts store. That is, the treating part 103 asks for the particle number contained in each division on size distribution figures based on particle size distribution.

[0033]In Step S6, the treating part 103 **********s the measurement count n. In Step S7, the treating part 103 judges whether the measurement count n is 2. If it is judged as "Yes", in order to perform 2nd measurement, it will shift to Step S8. If it is judged as "No", it will shift to step S9 mentioned later. Since measurement of the particle size distribution culture before and after culture is completed, it is for searching for the number of microorganism which increased from the difference of both particle size distribution.

[0034]In Step S8, the treating part 103 stands by that the predetermined time T passes. In order to change with samples, it is difficult to specify generally, but in the case of urine, this time is usually enough in about 4 hours, for example. In step S9 - Step S14, processing subdivided by said step S4 which asks for a changed part of division data and determines the number of increases of a bacillus for every division is performed.

[0035]First, in step S9, the treating part 103 sets up the division of a processing object out of the division of a predetermined number. Usually, order is used with a processing object from a division (x= 1, y= 1). In Step S10, about the division of a processing object, the treating part 103 subtracts the division data before culture from the division data after culture, and asks for a changed part of division data. In Step S11, the treating part 103 judges whether a changed part of division data is less than a predetermined value. If it is judged as "Yes", it will shift to Step S12. If it is judged as "No", it will shift to Step S13 mentioned later. When a changed part of division data is too much small, it is for considering that there is no number of increases of a bacillus in consideration of an error of measurement etc. A predetermined value is usually defined experientially.

[0036]In Step S12, the treating part 103 sets the number of increases of a bacillus as zero about the division data of a processing object. The treating part 103 matches a division and the number of increases of a bacillus, and stores them in the storage parts store 102. In Step S13, about the division of a processing object, since a changed part of division data is beyond a predetermined value, the treating part 103 is taken as = (the number of increases of a bacillus) (a changed part of division data). Like said step S12, the treating part 103 matches a division and the number of increases of a bacillus, and stores them in the storage parts store 102.

[0037]In Step S14, the treating part 103 judges whether it asked for the number of increases of the bacillus about all the divisions. If it is judged as "Yes", it will shift to Step S15. If it is

judged as "No", it will return to step S9 and the number of increases of a bacillus will be determined about the following division. In Step S15 - Step S20, processing which displays the particle size distribution before and behind culture and the analysis result of a bacillus which increased in order is performed. Only the bacillus which did not need to display these [all], for example, increased may be displayed. It is also possible to display each analysis result according to the directions from a user.

[0038]First, in Step S15, the treating part 103 determines whether to make which particle size distribution of before culture, after culture, or the bacillus that increased into analysis and a displaying object. In this example of an embodiment, it shall display before culture and after culture in order of the bacillus which increased. In Step S16, the treating part 103 searches for the rate of each division data when division data makes the division data of the division which is the maximum 100%. When it is the bacillus which the analytical object increased, the rate of the number of increases of the bacillus of each division when the number of increases of a bacillus makes 100% number of microorganism of the division which is the maximum is searched for.

[0039]In Step S17, the treating part 103 determines the division below a rate predetermined in the number of increases of division data or a bacillus as a display portion. This is because it will become difficult to distinguish the characteristic of the bacillus which particle size distribution became hard to see, and was increased if all the divisions are made into a displaying object. Drawing 21 mentioned later - drawing 44 show the analysis result when the rate of the number of increases of division data or a bacillus uses 10% or less of portion as a display portion.

[0040]In Step S18, the treating part 103 divides the rate of the number of increases of division data or a bacillus into the level defined beforehand, and sets up a different predetermined foreground color for every level about each division in a display portion. Drawing 21 mentioned later - drawing 44 are the examples of the analysis result displayed using a color different every five levels, 0 to 2%, 2 to 4%, 4 to 6%, 6 to 8%, and 8 to 10%.

[0041]In Step S19, the output control part 104 outputs a display portion to an outputting part using the set-up foreground color. Thereby, the analysis result illustrated to drawing 21 - 28 is displayed on an outputting part. In Step S20, the treating part 103 judges whether the analysis result was displayed about all after [before culture] culture or before after-culture-culture. Processing will be ended if it is judged as "Yes." If it is judged as "No", it will return to said step S15 again, and the analysis result which is not displayed will be displayed.

[0042][Example] -- the measurement of the microorganism in a sample performed using the account flow cytometer of before and a signal processor is explained.

(1) Urine was used as measurement (1-1) culture and the measurement sample of the number of microorganisms. The inspection of the bacteria in urine is widely conducted as a clinical laboratory test for diagnosis of urinary tract infection, such as cystitis and a **** nephritis.

[0043]As bacterial broth in urine, the heart yne FIJON bouillon (product made from NISSUI) which is a liquid medium for bacterium was used. following handling explanation as directions for use -- warming -- it was used after carrying out high-pressure steam sterilization of what was dissolved. First, two test tubes into which 2 ml of culture medium was put were prepared. 100micro of urine specimens l were added and stirred in each test tube, and two culture samples were prepared. Without carrying out culture processing of one of a culture sample, the microbial count was measured with flow cytometer so that it might mention later. After putting one more culture sample into the homoiothermal machine and cultivating at 37 ** for 4 hours, the microbial count was similarly measured with flow cytometer.

[0044]Measurement of the microbial count by a blow cytometer was performed as follows. Quantity of the sample analyzed with flow cytometer was set to 0.8microl. Flow cytometer

detected forward scattering light, detection light was inputted into the signal processor 10, and the forward scattering light intensity to forward scattering photoluminescence time was measured. The measurement result of the 1st particle size distribution and the culture sample before culture processing was made into the 2nd particle size distribution for the measurement result of the culture sample by which culture processing was carried out, and the final particle size distribution of the culture sample was searched for in quest of the difference of the 1st particle size distribution and the 2nd particle size distribution.
[0045]Drawing 6 and drawing 7 show the measurement result of the 1st and 2nd particle size distribution of a sample. A horizontal axis is the scattered-light emission time Fscw, and a vertical axis is the scattered light intensity Fsc. 100ch of the vertical axis of the particle size distribution shown in drawing 6 and drawing 7 is made into particle diameter, and is equivalent to about 1 micrometer. The particle size distribution of drawing 6 and drawing 7 showed that no less than 25676 particles increased. It asked for the increased particle number from the difference of the data number (the number of plots in each particle size distribution) before and after culture.
[0046](1-2) Comparison with the microbial biomass calculated from the accuracy comparison with the conventional measuring method, next the particle size distribution before and behind culture and the microbial biomass cultivated and measured using the conventional agar plate culture medium was performed. The sample was prepared and cultivated the same with having stated above (1-1), and particle size distribution was measured. It asked for the microbial count based on measurement (henceforth this method).
[0047]The culture and measurement by the agar plate culture medium made into a comparison object were performed as follows. First, the melon cult E (product made from Orion Diagnostica) usually used by the microorganism test in urine was used as an agar plate culture medium. This culture medium comprises a CLED culture medium, MacConkey's medium, and an enterococcus culture medium. According to the operation manual, the smear of the urine was carried out to the culture-medium surface, and culture was performed at 37 degrees for 24 hours. The judgment searched for the number of microorganism in urine for the density of the colony count visually as compared with the predetermined contrast table.
[0048]40 samples were followed in said melon cult E method and this method. A measurement result is shown in drawing 8. Drawing 8 shows the measurement result of the number of microorganism by the melon cult E method and this method. In the melon cult E method, more than bacterial count 10^5/ml is judged to be a positivity (bacteriuria), and bacterial count 10^4/ml is considered as the judgment suspension with positive doubt. Then, it compared with the measurement result of this method about 16 samples which became more than 10^4 individual / ml by the melon cult E method. The measurement result by this method showed the equivalent measurement result of more than 10^4 individual / ml also about which 16 samples. The difference of the measured value was within the limits of a single figure. From this, it can be said that the good correlation of the melon cult E method and this method was shown.
[0049]Next, while it was netative by the melon cult E method, by this method, it rechecked about the sample (drawing 8 Nakaya seal A) used as the positive finding of the high price. Then, there was a place which has melted into the culture-medium surface of the melon cult E. A colony of bacteria crowds too much and this happens. Although bacteria are increasing [this] in the melon cult E method about this sample, since a colony is not observed, it is thought that the erroneous decision was carried out to negativity.
[0050]Next, two samples (drawing 8 Nakaya seal B) from which this method became a low value compared with the melon cult E method were examined. Both the size distribution figures (drawing 9, drawing 11) after culture of both samples show the particle size distribution which spreads in the one where scattered-light emission time is longer. Since this

particle size distribution is not looked at by the size distribution figures (drawing 10, drawing 12) before culture, it is the particle size distribution of the bacillus increased by culture. Then, these culture samples were investigated still in detail under the microscope. A bacillus chaining each and increasing was observed in these samples. When the strain was furthermore investigated, the bacillus shown by drawing 9 and drawing 10 was Enterococcuserogenes of streptococci, and the bacillus shown by drawing 11 and drawing 12 was Pseudomonas of streptobacillus.

[0051]As mentioned above, the cause by which the measurement result of the melon cult E method and this method was not in agreement is considered as follows. Streptobacillus and streptococci are increased standing in a row in a chain. However, flow cytometer detects the chain lump which two or more bacilli chained as one particle. Then, the flow cytometer cannot detect the chained increment actual to it accumulating and being alike, even if growth of the bacillus has taken place.

[0052]Since streptobacillus and streptococci are the shape which stood in a row for a long time, scattered light intensity hardly changes, but according to the length of the bacillus which the emission time of the scattered light chained, it becomes long. Therefore, when scattered-light emission time supervises a long signal, growth of the streptobacillus and streptococci which are overlooked only with signal strength is detectable. If scattered-light emission time is the positivity in which streptobacillus or streptococci exist when the bacillus more than a predetermined number is detected to the field of 90 or more ch, specifically, it can judge. About the signal of the field, the increased microbial count can be presumed by multiplying by the coefficient made to correspond to the chained number of microorganism according to the length of scattered-light emission time.

[0053]It turned out that this method has the melon cult E method which is a conventional method, and good correlation, and a good measurement result can moreover be provided in culture time substantially shorter than before from the above examining result. It is also possible to specify the kind of microorganism by the same operation as the above by making the kind of culture medium into a selectivity culture medium. Since culture time can be managed in a short time, it becomes possible to change a culture condition and to examine a microorganism in detail for a short time.

[0054]The relation between vegetative form voice and size distribution figures was investigated about various kinds of microorganisms other than streptobacillus and streptococci. There is Staphylococcus as a bacillus which a bacillus serves as a group and is increased in addition to streptobacillus and streptococci. It cultivated like the above (1-1) by making two kinds of staphylococci into a sample, respectively, and flow cytometer detected the scattered light and the particle size distribution by a signal processor was measured. The size distribution figures are drawing 17 and drawing 18. Unlike the aforementioned chain bacillus, particle size distribution shows the particle size extended long and slender to the one where scattered light intensity is larger. Staphylococci increases in number, while bacilli gather like a fringe by growth. Therefore, according to growth of a bacillus, the path of the group of a bacillus becomes large and scattered light intensity also becomes large.

[0055]Even if the bacillus increased, the group investigated particle size distribution similarly about the Bacillus which is a bacillus not becoming. The size distribution figures are shown in drawing 15 and drawing 16. Particle size distribution which spreads in the one where the intensity and emission time of a scattered-light signal are larger is not shown. Since bacilli are in a scattering state even if a bacillus increases, this shows that it appears at the place below constant value, without the intensity and emission time of a scattered-light signal changing.

[0056]In flow cytometer, the above examination showed measuring lower than the number of growth actual about streptobacillus, streptococci, and Staphylococcus, in order to regard the

group of a bacillus as one particle. Since vegetative form voice differed, particle size distribution differed among streptobacillus, streptococci, Staphylococcus, and a Bacillus, and based on the particle size distribution of the increased bacillus, it turned out that they can specify the kind of bacillus. Especially, since streptobacillus and streptococci are seldom different from a Bacillus with independent scattered light intensity, distinction of them is impossible only by measuring scattered light intensity. By measuring two parameters of the intensity of a scattered-light signal, and emission time, it becomes possible to distinguish Staphylococcus, streptococci, streptobacillus, and a Bacillus. Drawing 45 shows typically the difference in the particle size distribution by said four sorts of bacilli.

[0057]when a bacillus is detected [scattered light intensity] for the emission time of the scattered light to the field of 90 or more ch by 90 or less ch, for example as for more than a predetermined number, streptobacillus specifically exists -- an alarm -- appearance matter -- things can be considered. When a bacillus is detected [scattered light intensity] for the emission time of the scattered light to the field of 80 or less ch by 50 or more ch as for more than a predetermined number, if Staphylococcus exists, it is possible to take out an alarm. Since particle size distribution is prolonged according to growth, streptobacillus and Staphylococcus can also supervise the shape of particle size distribution for a judgment only with the number of a field.

[0058]Since particle size distribution changes with collective number of microorganism, about the signal of the field, it is also possible to presume the increased number of microorganism itself by multiplying by the coefficient made to correspond to the number of microorganism for which it gathered according to the intensity of the scattered light, or the size of emission time. Since particle size distribution was measured also about yeast-like fungi, the result is shown. Drawing 13 and drawing 14 show the size distribution figures of yeast-like fungi. Since a signal appears at the place where scattered light intensity exceeded 250ch, the particle size has not appeared in this drawing. It is because the size of yeast-like fungi is as large as 3-5 micrometers, so scattered light intensity also becomes strong.

[0059](2) Since the measurement result was analyzed with the signal processor concerning the example of a classification book embodiment of a microorganism and the microorganism was classified, explain a measurement result and an analysis result. The following bacteria were used as a candidate for culture. The following bacteria are bacteria detected in urine, and when using urine for a sample and conducting a bacteriological examination, they are bacteria used as a subject of examination.

[0060]

Bacillus (1) Escherichia coli. Bacillus (2) Pseudomonas aeruginosa. Staphylococcus (1) Staphylococous. aureus Staphylococcus (2) Staphylococous epldermidis Streptococci (1) Entercococus faecalis Streptococci (2) Streptococous agalactlae. streptobacillus [ ] -- Pseudomonas yeast-like-fungi Candida glabrata -- the bacteria of these were cultivated like the above (1-1), flow cytometer detected the scattered light and the particle size distribution by a signal processor was measured. However, culture time experimented not as 4 hours but as 2 hours. Drawing 15 - 20 show the particle size distribution before culture of each bacteria, and after culture. As for Staphylococcus (1) and drawing 18, a Bacillus (1) and drawing 16 are [drawing 15 / a Bacillus (2) and drawing 17 / streptococci (1) and drawing 20 of Staphylococcus (2) and drawing 19] the particle size distribution about streptococci (2). In each figure, (a) shows the particle size distribution before culture, and (b) shows the particle size distribution after culture. The particle size distribution of streptobacillus is as being shown in said drawing 11 and 12. The particle size distribution of yeast-like fungi is as being shown in said drawing 13 and 14.

[0061]In each figure of said drawing 15 - 20, by comparing a figure (a) with a figure (b) shows in which field on size distribution figures bacteria are increasing. However, said

change is unclear when there is no remarkable change. Then, as mentioned above, it asked for a changed part of the particle size distribution before and after culture for every division of size distribution figures, and analysis processing as which the rate of change displays the division of a prescribed range was performed.

[0062]An analysis result is shown in drawing 21 - drawing 44. An analysis result is the graph which classified by color and displayed 10% or less of division at the time of dividing the graph of said particle size distribution into the division of 256x256, deducing the particle number in each division, and making most particle numbers into 100% for every predetermined level. Drawing 21, 22, and drawing 23 are analysis results which show the particle size distribution for its change before culture and after culture about a Bacillus (1), respectively. Drawing 24, 25, and drawing 26 are analysis results which show the particle size distribution for its change before culture and after culture about a Bacillus (2), respectively. The scattered light intensity of a Bacillus (1) is in the range of 10 - 80ch in general. The scattered light intensity of a Bacillus (2) is in the range of 0 - 20ch. The emission time of the scattered light is the range of 40 - 90ch in general in a Bacillus (1), and is as short as the range of 30 - 70ch at a Bacillus (2). Although an a few difference is in the position of particle size distribution according to the kind of Bacillus, particle size distribution is settled without seldom spreading. That is, not becoming a group even if it increases a Bacillus is shown. It is shown that a Bacillus (2) is a small Bacillus with small particle diameter.

[0063]Drawing 27, and 28 and 29 are analysis results which show the particle size distribution for its change before culture and after culture about Staphylococcus (1), respectively. Drawing 30, and 31 and 32 are analysis results which show the particle size distribution for its change before culture and after culture about Staphylococcus (2), respectively. Since the candidate for culture is Staphylococcus, it is crossing to the range with wide scattered light intensity of the increased bacillus.

[0064]For example, in Staphylococcus (1), the scattered light intensity of the increased bacillus is mainly crossed to the range of 20 - 190ch. In Staphylococcus (2), the scattered light intensity of the increased bacillus is mainly crossed to the range of 0 - 160ch. However, the emission time of the scattered light is in the range of 40 - 90ch, and it is large as scattered light intensity becomes large. That is, it is shown that the bacillus is increasing not to a chain but to tufted.

[0065]Drawing 33, and 34 and 35 are analysis results which show the particle size distribution for its change before culture and after culture about streptococci (1), respectively. Drawing 36, and 37 and 38 are analysis results which show the particle size distribution for its change before culture and after culture about streptococci (2), respectively. Since the candidates for culture are streptococci, the scattered light intensity of the increased bacillus has mainly been collected into the small range of 0 - 50ch. However, the emission time of the scattered light has spread irrespective of the size of scattered light intensity in the range of 30 - 170ch in 40 - 220ch and streptococci (2) with streptococci (1). That is, it is shown that it is chained although the increased bacillus is a byway, and length is long.

[0066]Drawing 39, and 40 and 41 are analysis results which show the particle size distribution for its change before culture and after culture about streptobacillus, respectively. Since the candidate for culture is streptobacillus, the scattered light intensity of the increased bacillus has mainly been collected into the small range of 30 - 60ch. However, the emission time of the scattered light has spread irrespective of the size of scattered light intensity in the range of 60 - 180ch. That is, a byway bacillus chaining and increasing is shown. each of streptococci and streptobacillus follows the chain degree of a bacillus -- scattered-light emission time -- **** -- it hears -- it becomes. However, since scattered light intensity is based on the path of each bacillus, since the path is larger than streptococci, also in scattered

light intensity, the direction of streptobacillus becomes large.
[0067]Drawing 42, and 43 and 44 are analysis results which show the particle size distribution for its change before culture and after culture about yeast-like fungi, respectively. Since the candidates for culture are yeast-like fungi, by a diagram, the scattered light intensity of the increased bacillus is near 240ch, but as for this, the large signal of 240 or more ch is included. Emission time is crossed to the range of 60 - 150ch.
[0068]As mentioned above, the user can guess [whether based on the analysis result of the bacillus increased by culture, the bacillus increased to any of five classifications, a Bacillus, Staphylococcus, streptococci, streptobacillus, and yeast-like fungi, corresponds, and]. It is also possible to judge to any of said five classifications the increased bacillus corresponds based on an analysis result with the signal processor 10. For example, the section area on the particle size distribution belonging to each of said five classifications is appointed beforehand, and it is possible to express a classification result according to the signal number of each section area. Analyzing the peak position and distribution width of grading analysis, and classifying a bacillus is also considered.
[0069]When a sample is urine, the most of the Bacillus and streptobacillus in urine is Gram negative, and, as for Staphylococcus and streptococci in urine, it is known that the most is a Gram positive. Therefore, it is possible to guess a classification of a bacillus based on the analysis result of said increased bacillus, and to presume to a Gram positive/negativity.
[0070]
[Effect of the Invention]By using this invention, using flow cytometer, a microorganism can be easily measured by short-time culture, the influence of impurity on a measurement result can be decreased, and exact measurement can be expected. From the emission time and signal strength of the scattered light which flow cytometer detects, the outline of a classification of a microorganism can be guessed and the time which an inspection takes can be shortened.

[Translation done.]

TECHNICAL FIELD

[Field of the Invention]This invention relates to the method of cultivating the microorganism in which bacteria and other culture are possible, and measuring number of microorganism. Especially, also in the sample in which impurity other than a microorganism is contained, it is related with the microorganism measuring method which measures number of microorganism to high sensitivity by short-time culture.

[Translation done.]

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PRIOR ART

[Description of the Prior Art]Conventionally, general methods of cultivating a microorganism and measuring the number of microorganisms include an agar plate smear method. In this method, the smear of a constant rate of samples is carried out on the agar plate culture medium containing a moderate nutrient, and after cultivating until a colony becomes a size observable under a naked eye or a microscope, the produced colony count is measured. However, if this method is used, it must wait for a bacillus colony to grow to the grade which becomes observable under a naked eye or a microscope. Therefore, in order to guess the kind of bacillus to some extent, the incubation period of about 18 to 24 hours is usually required. Depending on a strain, incubation period also has a thing over long periods of time, such as 24 hours or more, 48 hours or more, or January.

[0003]The drug susceptibility testing for mycobacterium which investigates the validity of the identification inspection of the strain for specifying a microorganism depending on the kind of bacillus increased by culture or drugs may be needed. However, it is difficult to take time in said method, before these necessity becomes clear, and to inspect many samples efficiently. There is a method of using a liquid medium instead of the above-mentioned agar plate culture medium, and cultivating a sample. This method cultivates by mixing a constant rate of samples with the liquid medium containing a moderate nutrient, and measures turbidity with a naked eye, an absorbance altimeter, a spectrophotometer, etc. However, since sensitivity says a photometer but there is, it must wait for growth of a bacillus till the grade from which a nephelometry changes also in this method. [no] Therefore, like an agar plate smear method, the incubation period for how many days is required for measurement of number of microorganism, and there is the above-mentioned problem in it.

[0004]The sample under culture is irradiated and the method of measuring number of microorganism from the change to the time of the scattered light and the transmitted light is indicated by JP,5-82901,A. Even if this method is a bacillus in which growth gestalten differ, it can search for number of microorganism correctly. However, in order to use the change of scattered-light data and the change of transmitted light data in the growth course of a bacillus for this method, the time which culture of a sample takes is not shortened.

[Translation done.]

EFFECT OF THE INVENTION

[Effect of the Invention]By using this invention, using flow cytometer, a microorganism can be easily measured by short-time culture, the influence of impurity on a measurement result can be decreased, and exact measurement can be expected. From the emission time and signal strength of the scattered light which flow cytometer detects, the outline of a classification of a microorganism can be guessed and the time which an inspection takes can be shortened.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention]Particle measuring devices, such as flow cytometer, are known as a device which, on the other hand, measures the particles of a minute size like a microorganism to high sensitivity. Since a particle measuring device measures the number of the particles in a sample one by one, high sensitivity measurement is possible for it. Therefore, if a particle measuring device is used, even if it does not carry out long term culture, the bacterial count in a sample is measurable.
[0006]However, a particle measuring device will detect impurity, such as the particles of size about the same as a microorganism, for example, dust, and a sludge, as a microorganism. For this reason, the error by impurity will be included in the detection result of a particle measuring device. For example, when measuring bacteria by making urine into a sample, red corpuscles, leucocytes, epithelial cells, a pillar, a crystal, etc. which are the physical components in the urine, and its collapse thing will be detected as bacteria.
[0007]Then, in order to identify impurity and a microorganism, a microorganism is dyed and the method of measuring the fluorescence shown from a microorganism is proposed. However, a dyeing degree changes with kinds of microorganism. Fluorescent dye and the processing condition which dye only the target microorganism must be set up for every

sample. For this reason, measurement takes time and effort and it is unsuitable for analyzing a lot of samples.
[0008]Otherwise, change of the impedance of the culture medium accompanying growth of a microorganism, change of PH of culture medium, the amount of consumption oxygen, or the amount of generating carbon dioxide is measured by making a microbial count into the method of detecting promptly, and the method of asking for a microbial count from correlation of these and a microbial count is studied these days. However, said measured value may change with causes other than the cultivated microorganism. Since the method of satisfying from the detection limit of a microorganism and a point of detecting accuracy cannot say, it is the method of using only by the specific condition lower. That is, the method of judging is not yet provided with the microorganism in a sample for a short time and and correctly as mentioned above.
[0009]This invention is a thing in consideration of the above situations, and an object of this invention is to provide the method of measuring measurement of a microorganism with simply sufficient accuracy by short-time culture.

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MEANS

[Means for Solving the Problem]In order that the 1st invention of this application may solve the above-mentioned technical problem, it is a measuring method of a microorganism in a culture sample which added a sample of a measuring object to culture medium, The 1st measuring process that measures said culture sample after carrying out A; predetermined time culture processing using said flow cytometer, and acquires the 1st particle size distribution of said culture sample from optical information of the scattered light detected, B; The 2nd measuring process that measures a culture sample before said culture processing using said flow cytometer, and acquires the 2nd particle size distribution of said culture sample from optical information of the scattered light detected, An analysis process which acquires particle size distribution of a microorganism cultivated in said culture sample from difference of the 1st particle size distribution of C; above, and the 2nd particle size distribution, and D; a microorganism measuring method including an output process which outputs particle size distribution of said obtained microorganism is provided.
[0011]By subtracting a measurement result before culture from a measurement result after culture, it can prevent measuring as a microorganism impurity contained in a sample. Impurity is a physical component in dust or a sample, for example. In said 1st invention, the 2nd invention of this application measures emission time and intensity of a scattered-light signal which are detected using said flow cytometer, and provides a microorganism measuring method acquiring said 1st and 2nd particle size distribution.

[0012]Scattered light intensity gives information about a size of grain contained in a culture sample. Scattered-light emission time gives information about the length of grain contained in a culture sample. Therefore, vegetative form voice of increasing to a chain or increasing to tufted [of a grape] can be known by measuring change of intensity to emission time. The 3rd invention of this application by measuring emission time and intensity of a scattered-light signal which are detected using said flow cytometer in said 1st invention, Particle size distribution of said cultivated microorganism is acquired, vegetative form voice of said microorganism is presumed based on said particle size distribution, and a microorganism measuring method judging to any of a predetermined classification said microorganism belongs is provided.
[0013]When a bacillus whose length whose path is comparatively large and is not so long is increasing, Staphylococcus, Streptococci can be presumed when a bacillus with them is increasing. [streptobacillus, a path still smaller than streptobacillus, and] [long when a bacillus with them is increasing] [a Bacillus, a comparatively small path, and] [long when a bacillus with short length whose path is comparatively small is increasing] The 4th invention of this application acquires particle size distribution of said cultivated microorganism by measuring emission time and intensity of a scattered-light signal which are detected using said flow cytometer in said 1st invention, A microorganism measuring method judging to any vegetative form voice of said microorganism shall belong between Staphylococcus, streptococci, streptobacillus, or a Bacillus based on said particle size distribution is provided.
[0014]It has the same operation effect as said 3rd invention. The 5th invention of this application provides a microorganism measuring method, wherein a measuring object sample is urine in said 1st invention. When making urine into a sample, a classification result of said microorganism to Gram negative / positive judgment is possible.
[0015]The 6th invention of this application provides a microorganism measuring method characterized by measuring bacteria and/or yeast-like fungi as a microorganism in said 1st invention. The 7th invention of this application is used with flow cytometer which measures a particle size of a microorganism in a culture sample which added a measuring object sample to culture medium, and provides a microorganism measuring device provided with the 1st measuring means, the 2nd measuring means, an analysis means, and an output means. The 1st measuring means acquires the 1st particle size distribution of a culture sample after performing predetermined time culture processing from optical information of the scattered light detected by said flow cytometer. The 2nd measuring means acquires the 2nd particle size distribution of a culture sample before said culture processing from optical information of the scattered light detected by said flow cytometer. An analysis means acquires particle size distribution of a microorganism cultivated in said culture sample from difference of said 1st particle size distribution and the 2nd particle size distribution. An output means outputs particle size distribution of said obtained microorganism.
[0016]It has the same operation effect as said 1st invention. The 8th invention of this application is used with flow cytometer which measures a particle size of a microorganism in a culture sample which added a measuring object sample to culture medium, It is the recording medium which recorded an analysis program which analyzes a measurement result of said particle size and in which computer reading is possible, and a recording medium which recorded an analysis program for performing the following A - D stage and in which computer reading is possible is provided.
A; from optical information of the scattered light detected by said flow cytometer. A stage of acquiring the 1st particle size distribution of a culture sample after performing predetermined time culture processing, B; from optical information of the scattered light detected by said flow cytometer. A stage, D which acquire particle size distribution of a microorganism cultivated in said culture sample based on difference of a stage, the 1st particle size

distribution of C; above, and the 2nd particle size distribution which acquire the 2nd particle size distribution of a culture sample before said culture processing; a stage which outputs particle size distribution of said obtained microorganism.
[0017]It has the same operation effect as said 1st invention.
[0018]
[Embodiment of the Invention]Hereafter, this invention is explained concretely, giving the example of an embodiment. In this invention, by wrapping and passing a sample with sheath liquid, flow cytometer makes the thin sample flow by the hydrodynamics effect form, passes one particle in b sample at a time to a primary detecting element, and means the device which detects the scattered light which irradiates with c light and is emitted from particles, and fluorescence. As a light source, a semiconductor laser, argon laser, etc. can be used suitably.
[0019]The culture medium refers to the liquid medium containing the moderate nutrient for cultivating a microorganism. If a microorganism kind is not specified, the culture medium which was suitable for the kind in the culture medium for bacterium when the microorganism kind was specified can be used selectively. Culture processing is processing for cultivating a microorganism, a culture sample is put into the humidistat of a predetermined temperature (for example, 30-37 **), and the processing which promotes culture of a microorganism is said.
[0020]It is measuring the microorganism in the culture sample before culture processing is performed in the culture sample before culture processing as for measurement. Measuring, after saving a culture sample at low temperature which a microorganism does not increase also contains.
Below the <example of a 1st embodiment> explains this invention in full detail based on the example of an embodiment shown in a drawing. This invention is not limited by this.
[0021][Device] Drawing 1 is a lineblock diagram containing the primary detecting element of the flow cytometer of this invention, and a signal processor. The primary detecting element of flow cytometer has the sheath flow cell 1, the sample nozzle 2, the beam stopper 5, the collector lens 6, the dichroic filter 7, the photo-diode 8, and the photograph mull 9.
[0022]The sheath flow cell 1 forms the sample flow 4 which wrapped the sample which flows from the sample nozzle 2 in sheath liquid, and was wrapped in sheath liquid. The particles 4 of sample flow can irradiate with the laser beam 3 from the laser source which is not illustrated. The beam stopper 5 intercepts the light which penetrates the sample flow 4 directly. The collector lens 6 condenses the forward scattering light and the front fluorescence which the particles 4 emit. The dichroic filter 7 reflects forward scattering light. The photo-diode 8 detects the forward scattering light reflected by the dichroic filter 7. The detected forward scattering light is inputted into the signal processor 10 via amplifier. The photograph mull 9 detects the front fluorescence which passed the dichroic filter 7. The forward scattering light and the front fluorescence which were detected by the photo-diode 8 and the photograph mull 9 are inputted into the signal processor 10 via amplifier, respectively.
[0023]The signal processor 10 concerning this invention receives the detecting signal outputted from the photo-diode 8, Change of the scattered light intensity (Fsc;Forwardscatter intensity) to the emission time (Fscw;Forward scatter pulse width) of forward scattering light is measured. The signal processor 10 creates and analyzes size distribution figures based on a forward scattering lightwave signal. The key map of a signal inputted into the signal processor 10 is shown in drawing 2. Scattered light intensity is equivalent to the intensity, i.e., the pulse height, of an input signal. The emission time of the scattered light is equivalent to the pulse width of an input signal.
[0024]Drawing 3 is a block diagram showing the functional constitution of the signal processor 10. The signal processor 10 is formed, for example in information terminals, such as PC (Personal Computer) and WS (WorkStation), and outputs a processing result to the

outputting part of an information terminal. The signal processor 10 is provided with the particle-size-distribution preparing part 101, the storage parts store 102, the treating part 103, and the output control part 104.
[0025]The particle-size-distribution preparing part 101 receives the lightwave signal which flow cytometer detected, and creates the particle size distribution which makes a horizontal axis forward scattering photoluminescence time, and makes a vertical axis forward scattering photoluminescence intensity based on an input signal. An output is possible for the particle-size-distribution preparing part 101 to outputting parts, such as a display, via the output control part 104 in the size distribution figures showing the created particle size distribution. Drawing 6 mentioned later, drawing 7, etc. are examples of the size distribution figures outputted by the particle-size-distribution preparing part 101.
[0026]The storage parts store 102 memorizes the created size distribution figures about each of after culture and a front. The storage parts store 102 memorizes the analysis result of division data and size distribution figures which are created based on size distribution figures. Division data and an analysis result are mentioned later. The treating part 103 subdivides size distribution figures to a predetermined division, and asks for the data number (only henceforth division data) in each division. In other words, division data is a particle number contained in one division on size distribution figures. The treating part 103 asks for a changed part of the division data in the same division in the particle size distribution of the back before culture. Thereby, the error of measurement by measuring the impurity in a sample as a microorganism can be decreased.
[0027]The error of measurement by impurity is explained. 1 micrometer or less of impurity of size about the same as the microorganism in culture medium also detects the detection by the forward scattering light intensity of flow cytometer. Many particles are detected also before culture processing (refer to drawing 6 mentioned later). Even if many particles are detected after culture processing, it cannot be judged whether it is the impurity which is mixing from the beginning whether it is the microorganism which it increased. Then, the particle size distribution before culture processing is subtracted from the particle size distribution after culture processing, and the particles which increased by culture processing, i.e., the cultivated microorganism, are detected.
[0028]Based on a part for each division data or the change, the treating part 103 analyzes particle size distribution by a predetermined method, and outputs an analysis result to a display control part. For example, when a division with most changed parts is made into 100%, the changed rate of each division data is searched for, and using the division below a predetermined rate as a display portion is mentioned. The treating part 103 sets the color according to the degree of change as each division, in order to display the degree of change of division data visually. The treating part 103 may set the color according to the size of division data as each division similarly about the particle size distribution of the back before culture.
[0029]The output control part 104 outputs each division to outputting parts, such as a display and a printer, using the foreground color set up by the treating part 103. Drawing 21 mentioned later - 28 are the display examples of the analysis result outputted by the output control part 104. About scattered light intensity, the horizontal axis of a vertical axis is the emission time of the scattered light. For example, drawing 21 shows about a Bacillus the analysis result of the particle size distribution of the microorganism which increased by the particle size distribution before culture, the particle size distribution after culture, and culture, respectively.
[0030][Flow of processing] Next, the flow of the analysis processing which the signal processor 10 performs is explained. Drawing 4 is a flow chart which shows an example of the flow of the analysis processing which the signal processor 10 performs. The following

processings are started by inputting a detecting signal from flow cytometer. First, in Step S1, the treating part 103 performs predetermined initialization processing. Specifically, the treating part 103 is set to n= 1, x= 1, and y= 1. Here, n is a variable which shows a measurement count. x and y are variables which show the subdivided position of x shaft orientations of the division of size distribution figures, and y shaft orientations.

[0031]In Step S2, the particle-size-distribution preparing part 101 receives a detecting signal from flow cytometer. In Step S3, the particle-size-distribution preparing part 101 asks for the luminescence intensity to the emission time of forward scattering light based on the received detecting signal, and creates particle size distribution. Furthermore, the particle-size-distribution preparing part 101 stores said created particle size distribution in the storage parts store 102. The particle-size-distribution preparing part 101 reads particle size distribution from the storage parts store 102 automatically, corresponding to the directions from a user, and an output is possible for it on a display etc.

[0032]In step S4, the treating part 103 subdivides size distribution figures in the predetermined number of divisions. For example, it subdivides to the division (1<=x<=256, 1<=y<=256) of 256x256. In Step S5, the treating part 103 asks for the division data for every division about all the divisions, and stores it in a storage parts store. That is, the treating part 103 asks for the particle number contained in each division on size distribution figures based on particle size distribution.

[0033]In Step S6, the treating part 103 **********s the measurement count n. In Step S7, the treating part 103 judges whether the measurement count n is 2. If it is judged as "Yes", in order to perform 2nd measurement, it will shift to Step S8. If it is judged as "No", it will shift to step S9 mentioned later. Since measurement of the particle size distribution culture before and after culture is completed, it is for searching for the number of microorganism which increased from the difference of both particle size distribution.

[0034]In Step S8, the treating part 103 stands by that the predetermined time T passes. In order to change with samples, it is difficult to specify generally, but in the case of urine, this time is usually enough in about 4 hours, for example. In step S9 - Step S14, processing subdivided by said step S4 which asks for a changed part of division data and determines the number of increases of a bacillus for every division is performed.

[0035]First, in step S9, the treating part 103 sets up the division of a processing object out of the division of a predetermined number. Usually, order is used with a processing object from a division (x= 1, y= 1). In Step S10, about the division of a processing object, the treating part 103 subtracts the division data before culture from the division data after culture, and asks for a changed part of division data. In Step S11, the treating part 103 judges whether a changed part of division data is less than a predetermined value. If it is judged as "Yes", it will shift to Step S12. If it is judged as "No", it will shift to Step S13 mentioned later. When a changed part of division data is too much small, it is for considering that there is no number of increases of a bacillus in consideration of an error of measurement etc. A predetermined value is usually defined experientially.

[0036]In Step S12, the treating part 103 sets the number of increases of a bacillus as zero about the division data of a processing object. The treating part 103 matches a division and the number of increases of a bacillus, and stores them in the storage parts store 102. In Step S13, about the division of a processing object, since a changed part of division data is beyond a predetermined value, the treating part 103 is taken as = (the number of increases of a bacillus) (a changed part of division data). Like said step S12, the treating part 103 matches a division and the number of increases of a bacillus, and stores them in the storage parts store 102.

[0037]In Step S14, the treating part 103 judges whether it asked for the number of increases of the bacillus about all the divisions. If it is judged as "Yes", it will shift to Step S15. If it is

judged as "No", it will return to step S9 and the number of increases of a bacillus will be determined about the following division. In Step S15 - Step S20, processing which displays the particle size distribution before and behind culture and the analysis result of a bacillus which increased in order is performed. Only the bacillus which did not need to display these [all], for example, increased may be displayed. It is also possible to display each analysis result according to the directions from a user.

[0038]First, in Step S15, the treating part 103 determines whether to make which particle size distribution of before culture, after culture, or the bacillus that increased into analysis and a displaying object. In this example of an embodiment, it shall display before culture and after culture in order of the bacillus which increased. In Step S16, the treating part 103 searches for the rate of each division data when division data makes the division data of the division which is the maximum 100%. When it is the bacillus which the analytical object increased, the rate of the number of increases of the bacillus of each division when the number of increases of a bacillus makes 100% number of microorganism of the division which is the maximum is searched for.

[0039]In Step S17, the treating part 103 determines the division below a rate predetermined in the number of increases of division data or a bacillus as a display portion. This is because it will become difficult to distinguish the characteristic of the bacillus which particle size distribution became hard to see, and was increased if all the divisions are made into a displaying object. Drawing 21 mentioned later - drawing 44 show the analysis result when the rate of the number of increases of division data or a bacillus uses 10% or less of portion as a display portion.

[0040]In Step S18, the treating part 103 divides the rate of the number of increases of division data or a bacillus into the level defined beforehand, and sets up a different predetermined foreground color for every level about each division in a display portion. Drawing 21 mentioned later - drawing 44 are the examples of the analysis result displayed using a color different every five levels, 0 to 2%, 2 to 4%, 4 to 6%, 6 to 8%, and 8 to 10%.

[0041]In Step S19, the output control part 104 outputs a display portion to an outputting part using the set-up foreground color. Thereby, the analysis result illustrated to drawing 21 - 28 is displayed on an outputting part. In Step S20, the treating part 103 judges whether the analysis result was displayed about all after [before culture] culture or before after-culture-culture. Processing will be ended if it is judged as "Yes." If it is judged as "No", it will return to said step S15 again, and the analysis result which is not displayed will be displayed.

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EXAMPLE

[Example] -- the measurement of the microorganism in a sample performed using the account flow cytometer of before and a signal processor is explained.
(1) Urine was used as measurement (1-1) culture and the measurement sample of the number of microorganisms. The inspection of the bacteria in urine is widely conducted as a clinical laboratory test for diagnosis of urinary tract infection, such as cystitis and a **** nephritis.
[0043]As bacterial broth in urine, the heart yne FIJON bouillon (product made from NISSUI) which is a liquid medium for bacterium was used. following handling explanation as directions for use -- warming -- it was used after carrying out high-pressure steam sterilization of what was dissolved. First, two test tubes into which 2 ml of culture medium was put were prepared. 100micro of urine specimens l were added and stirred in each test tube, and two culture samples were prepared. Without carrying out culture processing of one of a culture sample, the microbial count was measured with flow cytometer so that it might mention later. After putting one more culture sample into the homoiothermal machine and cultivating at 37 ** for 4 hours, the microbial count was similarly measured with flow cytometer.
[0044]Measurement of the microbial count by a blow cytometer was performed as follows. Quantity of the sample analyzed with flow cytometer was set to 0.8microl. Flow cytometer detected forward scattering light, detection light was inputted into the signal processor 10, and the forward scattering light intensity to forward scattering photoluminescence time was measured. The measurement result of the 1st particle size distribution and the culture sample before culture processing was made into the 2nd particle size distribution for the measurement result of the culture sample by which culture processing was carried out, and the final particle size distribution of the culture sample was searched for in quest of the difference of the 1st particle size distribution and the 2nd particle size distribution.
[0045]Drawing 6 and drawing 7 show the measurement result of the 1st and 2nd particle size distribution of a sample. A horizontal axis is the scattered-light emission time Fscw, and a vertical axis is the scattered light intensity Fsc. 100ch of the vertical axis of the particle size distribution shown in drawing 6 and drawing 7 is made into particle diameter, and is equivalent to about 1 micrometer. The particle size distribution of drawing 6 and drawing 7 showed that no less than 25676 particles increased. It asked for the increased particle number from the difference of the data number (the number of plots in each particle size distribution) before and after culture.
[0046](1-2) Comparison with the microbial biomass calculated from the accuracy comparison with the conventional measuring method, next the particle size distribution before and behind culture and the microbial biomass cultivated and measured using the conventional agar plate culture medium was performed. The sample was prepared and cultivated the same with having stated above (1-1), and particle size distribution was measured. It asked for the microbial count based on measurement (henceforth this method).
[0047]The culture and measurement by the agar plate culture medium made into a comparison object were performed as follows. First, the melon cult E (product made from Orion Diagnostica) usually used by the microorganism test in urine was used as an agar plate culture medium. This culture medium comprises a CLED culture medium, MacConkey's medium, and an enterococcus culture medium. According to the operation manual, the smear of the urine was carried out to the culture-medium surface, and culture was performed at 37 degrees for 24 hours. The judgment searched for the number of microorganism in urine for the density of the colony count visually as compared with the predetermined contrast table.
[0048]40 samples were followed in said melon cult E method and this method. A measurement result is shown in drawing 8. Drawing 8 shows the measurement result of the number of microorganism by the melon cult E method and this method. In the melon cult E method, more than bacterial count 10^5/ml is judged to be a positivity (bacteriuria), and

bacterial count 10^4/ml is considered as the judgment suspension with positive doubt. Then, it compared with the measurement result of this method about 16 samples which became more than 10^4 individual / ml by the melon cult E method. The measurement result by this method showed the equivalent measurement result of more than 10^4 individual / ml also about which 16 samples. The difference of the measured value was within the limits of a single figure. From this, it can be said that the good correlation of the melon cult E method and this method was shown.

[0049]Next, while it was netative by the melon cult E method, by this method, it rechecked about the sample (drawing 8 Nakaya seal A) used as the positive finding of the high price. Then, there was a place which has melted into the culture-medium surface of the melon cult E. A colony of bacteria crowds too much and this happens. Although bacteria are increasing [this] in the melon cult E method about this sample, since a colony is not observed, it is thought that the erroneous decision was carried out to negativity.

[0050]Next, two samples (drawing 8 Nakaya seal B) from which this method became a low value compared with the melon cult E method were examined. Both the size distribution figures (drawing 9, drawing 11) after culture of both samples show the particle size distribution which spreads in the one where scattered-light emission time is longer. Since this particle size distribution is not looked at by the size distribution figures (drawing 10, drawing 12) before culture, it is the particle size distribution of the bacillus increased by culture. Then, these culture samples were investigated still in detail under the microscope. A bacillus chaining each and increasing was observed in these samples. When the strain was furthermore investigated, the bacillus shown by drawing 9 and drawing 10 was Enterococcuserogenes of streptococci, and the bacillus shown by drawing 11 and drawing 12 was Pseudomonas of streptobacillus.

[0051]As mentioned above, the cause by which the measurement result of the melon cult E method and this method was not in agreement is considered as follows. Streptobacillus and streptococci are increased standing in a row in a chain. However, flow cytometer detects the chain lump which two or more bacilli chained as one particle. Then, the flow cytometer cannot detect the chained increment actual to it accumulating and being alike, even if growth of the bacillus has taken place.

[0052]Since streptobacillus and streptococci are the shape which stood in a row for a long time, scattered light intensity hardly changes, but according to the length of the bacillus which the emission time of the scattered light chained, it becomes long. Therefore, when scattered-light emission time supervises a long signal, growth of the streptobacillus and streptococci which are overlooked only with signal strength is detectable. If scattered-light emission time is the positivity in which streptobacillus or streptococci exist when the bacillus more than a predetermined number is detected to the field of 90 or more ch, specifically, it can judge. About the signal of the field, the increased microbial count can be presumed by multiplying by the coefficient made to correspond to the chained number of microorganism according to the length of scattered-light emission time.

[0053]It turned out that this method has the melon cult E method which is a conventional method, and good correlation, and a good measurement result can moreover be provided in culture time substantially shorter than before from the above examining result. It is also possible to specify the kind of microorganism by the same operation as the above by making the kind of culture medium into a selectivity culture medium. Since culture time can be managed in a short time, it becomes possible to change a culture condition and to examine a microorganism in detail for a short time.

[0054]Relation between vegetative form voice and size distribution figures was investigated about various kinds of microorganisms other than streptobacillus and streptococci. There is Staphylococcus as a bacillus which a bacillus serves as a group and is increased in addition to

streptobacillus and streptococci. It cultivated like the above (1-1) by making two kinds of staphylococci into a sample, respectively, and flow cytometer detected the scattered light and particle size distribution by a signal processor was measured. The size distribution figures are drawing 17 and drawing 18. Unlike the aforementioned chain bacillus, particle size distribution shows a particle size extended long and slender to the one where scattered light intensity is larger. Staphylococci increases in number, while bacilli gather like a fringe by growth. Therefore, according to growth of a bacillus, a path of a group of a bacillus becomes large and scattered light intensity also becomes large.

[0055]Even if a bacillus increased, a group investigated particle size distribution similarly about a Bacillus which is a bacillus not becoming. The size distribution figures are shown in drawing 15 and drawing 16. Particle size distribution which spreads in the one where intensity and emission time of a scattered-light signal are larger is not shown. Since bacilli are in a scattering state even if a bacillus increases, this shows that it appears at a place below constant value, without intensity and emission time of a scattered-light signal changing.

[0056]In flow cytometer, the above examination showed measuring lower than the number of growth actual about streptobacillus, streptococci, and Staphylococcus, in order to regard the group of a bacillus as one particle. Since vegetative form voice differed, particle size distribution differed among streptobacillus, streptococci, Staphylococcus, and a Bacillus, and based on the particle size distribution of the increased bacillus, it turned out that they can specify the kind of bacillus. Especially, since streptobacillus and streptococci are seldom different from a Bacillus with independent scattered light intensity, distinction of them is impossible only by measuring scattered light intensity. By measuring two parameters of the intensity of a scattered-light signal, and emission time, it becomes possible to distinguish Staphylococcus, streptococci, streptobacillus, and a Bacillus. Drawing 45 shows typically the difference in the particle size distribution by said four sorts of bacilli.

[0057]when a bacillus is detected [scattered light intensity] for the emission time of the scattered light to the field of 90 or more ch by 90 or less ch, for example as for more than a predetermined number, streptobacillus specifically exists -- an alarm -- appearance matter -- things can be considered. When a bacillus is detected [scattered light intensity] for the emission time of the scattered light to the field of 80 or less ch by 50 or more ch as for more than a predetermined number, if Staphylococcus exists, it is possible to take out an alarm. Since particle size distribution is prolonged according to growth, streptobacillus and Staphylococcus can also supervise the shape of particle size distribution for a judgment only with the number of a field.

[0058]Since particle size distribution changes with collective number of microorganism, about the signal of the field, it is also possible to presume the increased number of microorganism itself by multiplying by the coefficient made to correspond to the number of microorganism for which it gathered according to the intensity of the scattered light, or the size of emission time. Since particle size distribution was measured also about yeast-like fungi, the result is shown. Drawing 13 and drawing 14 show the size distribution figures of yeast-like fungi. Since a signal appears at the place where scattered light intensity exceeded 250ch, the particle size has not appeared in this drawing. It is because the size of yeast-like fungi is as large as 3-5 micrometers, so scattered light intensity also becomes strong.

[0059](2) Since the measurement result was analyzed with the signal processor concerning the example of a classification book embodiment of a microorganism and the microorganism was classified, explain a measurement result and an analysis result. The following bacteria were used as a candidate for culture. The following bacteria are bacteria detected in urine. When using urine for a sample and conducting a bacteriological examination, they are the bacteria used as a subject of examination.

[0060]
Bacillus (1) Escherichia coli. Bacillus (2) Pseudomonas aeruginosa. Staphylococcus (1) Staphylococous. aureus Staphylococcus (2) Staphylococous epldermidis Streptococci (1) Entercococus faecalis Streptococci (2) Streptococous agalactlae. streptobacillus [ ] -- Pseudomonas yeast-like-fungi Candida glabrata -- bacteria of these were cultivated like the above (1-1), flow cytometer detected the scattered light and particle size distribution by a signal processor was measured. However, culture time experimented not as 4 hours but as 2 hours. Drawing 15 - 20 show particle size distribution before culture of each bacteria, and after culture. As for Staphylococcus (1) and drawing 18, a Bacillus (1) and drawing 16 are [drawing 15 / a Bacillus (2) and drawing 17 / streptococci (1) and drawing 20 of Staphylococcus (2) and drawing 19] the particle size distribution about streptococci (2). In each figure, (a) shows particle size distribution before culture, and (b) shows particle size distribution after culture. Particle size distribution of streptobacillus is as being shown in said drawing 11 and 12. Particle size distribution of yeast-like fungi is as being shown in said drawing 13 and 14.
[0061]In each figure of said drawing 15 - 20, by comparing a figure (a) with a figure (b) shows in which field on size distribution figures bacteria are increasing. However, said change is unclear when there is no remarkable change. Then, as mentioned above, it asked for a changed part of the particle size distribution before and after culture for every division of size distribution figures, and analysis processing as which the rate of change displays the division of a prescribed range was performed.
[0062]An analysis result is shown in drawing 21 - drawing 44. An analysis result is the graph which classified by color and displayed 10% or less of division at the time of dividing the graph of said particle size distribution into the division of 256x256, deducing the particle number in each division, and making most particle numbers into 100% for every predetermined level. Drawing 21, 22, and drawing 23 are analysis results which show the particle size distribution for its change before culture and after culture about a Bacillus (1), respectively. Drawing 24, 25, and drawing 26 are analysis results which show the particle size distribution for its change before culture and after culture about a Bacillus (2), respectively. The scattered light intensity of a Bacillus (1) is in the range of 10 - 80ch in general. The scattered light intensity of a Bacillus (2) is in the range of 0 - 20ch. The emission time of the scattered light is the range of 40 - 90ch in general in a Bacillus (1). It is as short as the range of 30 - 70ch at a Bacillus (2).
Although an a few difference is in the position of particle size distribution according to the kind of Bacillus, particle size distribution is settled without seldom spreading. That is, not becoming a group even if it increases a Bacillus is shown. It is shown that a Bacillus (2) is a small Bacillus with small particle diameter.
[0063]Drawing 27, and 28 and 29 are analysis results which show particle size distribution for its change before culture and after culture about Staphylococcus (1), respectively. Drawing 30, and 31 and 32 are analysis results which show particle size distribution for its change before culture and after culture about Staphylococcus (2), respectively. Since a candidate for culture is Staphylococcus, it is crossing to a range with wide scattered light intensity of an increased bacillus.
[0064]For example, in Staphylococcus (1), scattered light intensity of an increased bacillus is mainly crossed to the range of 20 - 190ch. In Staphylococcus (2), scattered light intensity of an increased bacillus is mainly crossed to the range of 0 - 160ch. However, emission time of the scattered light is in the range of 40 - 90ch, and it is large as scattered light intensity becomes large. That is, it is shown that a bacillus is increasing not to a chain but to tufted.
[0065]Drawing 33, and 34 and 35 are analysis results which show particle size distribution for its change before culture and after culture about streptococci (1), respectively. Drawing

36, and 37 and 38 are analysis results which show particle size distribution for its change before culture and after culture about streptococci (2), respectively. Since candidates for culture are streptococci, scattered light intensity of an increased bacillus has mainly been collected into the small range of 0 - 50ch. However, emission time of the scattered light has spread irrespective of a size of scattered light intensity in the range of 30 - 170ch in 40 - 220ch and streptococci (2) with streptococci (1). That is, it is shown that it is chained although an increased bacillus is a byway, and length is long.

[0066]Drawing 39, and 40 and 41 are analysis results which show the particle size distribution for its change before culture and after culture about streptobacillus, respectively. Since the candidate for culture is streptobacillus, the scattered light intensity of the increased bacillus has mainly been collected into the small range of 30 - 60ch. However, the emission time of the scattered light has spread irrespective of the size of scattered light intensity in the range of 60 - 180ch. That is, a byway bacillus chaining and increasing is shown. each of streptococci and streptobacillus follows the chain degree of a bacillus -- scattered-light emission time -- **** -- it hears -- it becomes. However, since scattered light intensity is based on the path of each bacillus, since the path is larger than streptococci, also in scattered light intensity, the direction of streptobacillus becomes large.

[0067]Drawing 42, and 43 and 44 are analysis results which show the particle size distribution for its change before culture and after culture about yeast-like fungi, respectively. Since the candidates for culture are yeast-like fungi, by a diagram, the scattered light intensity of the increased bacillus is near 240ch, but as for this, the large signal of 240 or more ch is included. Emission time is crossed to the range of 60 - 150ch.

[0068]As mentioned above, the user can guess [whether based on the analysis result of the bacillus increased by culture, the bacillus increased to any of five classifications, a Bacillus, Staphylococcus, streptococci, streptobacillus, and yeast-like fungi, corresponds, and]. It is also possible to judge to any of said five classifications the increased bacillus corresponds based on an analysis result with the signal processor 10. For example, the section area on the particle size distribution belonging to each of said five classifications is appointed beforehand, and it is possible to express a classification result according to the signal number of each section area. Analyzing the peak position and distribution width of grading analysis, and classifying a bacillus is also considered.

[0069]When a sample is urine, the most of the Bacillus and streptobacillus in urine is Gram negative, and, as for Staphylococcus and streptococci in urine, it is known that the most is a Gram positive. Therefore, it is possible to guess a classification of a bacillus based on the analysis result of said increased bacillus, and to presume to a Gram positive/negativity.

[Translation done.]

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DESCRIPTION OF DRAWINGS

[Brief Description of the Drawings]
[Drawing 1]The explanatory view showing the composition of the primary detecting element of flow cytometer.
[Drawing 2]The signal-description figure inputted into flow cytometer.
[Drawing 3]The block diagram showing the functional constitution of a signal processor.
[Drawing 4]The flow chart which shows the flow of the analysis processing which a signal processor performs (first half).
[Drawing 5]The flow chart which shows the flow of the analysis processing which a signal processor performs (second half).
[Drawing 6]Size distribution figures after culture processing (urine).
[Drawing 7]Size distribution figures before sample culture processing of drawing 3.
[Drawing 8]The measurement result of the melon cult E method and this method.
[Drawing 9]Size distribution figures after culture processing of streptococci.
[Drawing 10]Size distribution figures before culture processing of the streptococci of drawing 6.
[Drawing 11]Size distribution figures after culture processing of streptobacillus.
[Drawing 12]Size distribution figures before culture processing of the streptobacillus of drawing 11.
[Drawing 13]Size distribution figures after culture processing of yeast-like fungi.
[Drawing 14]Size distribution figures before culture processing of the yeast-like fungi of drawing 10.
[Drawing 15](a) Particle size distribution of the Bacillus (1) before culture
(b) Particle size distribution of the Bacillus (1) after culture
[Drawing 16](a) Particle size distribution of the Bacillus (2) before culture
(b) Particle size distribution of the Bacillus (2) after culture
[Drawing 17](a) Particle size distribution of Staphylococcus (1) before culture
(b) Particle size distribution of Staphylococcus (1) after culture
[Drawing 18](a) Particle size distribution of Staphylococcus (2) before culture
(b) Particle size distribution of Staphylococcus (2) after culture
[Drawing 19](a) Particle size distribution of the streptococci (1) before culture
(b) Particle size distribution of the streptococci (1) after culture
[Drawing 20](a) Particle size distribution of the streptococci (2) before culture
(b) Particle size distribution of the streptococci (3) after culture
[Drawing 21]The analysis result of the particle size distribution of a Bacillus (1) (before culture).
[Drawing 22]The analysis result of the particle size distribution of a Bacillus (1) (after culture).
[Drawing 23]The analysis result of the particle size distribution of a Bacillus (1) (before after-culture -).
[Drawing 24]The analysis result of the particle size distribution of a Bacillus (2) (before culture).
[Drawing 25]The analysis result of the particle size distribution of a Bacillus (2) (after culture).
[Drawing 26]The analysis result of the particle size distribution of a Bacillus (2) (before after-culture -).
[Drawing 27]The analysis result of the particle size distribution of Staphylococcus (1) (before culture).
[Drawing 28]The analysis result of the particle size distribution of Staphylococcus (1) (after culture).

[Drawing 29]The analysis result of the particle size distribution of Staphylococcus (1) (before after-culture -).
[Drawing 30]The analysis result of the particle size distribution of Staphylococcus (2) (before culture).
[Drawing 31]The analysis result of the particle size distribution of Staphylococcus (2) (after culture).
[Drawing 32]The analysis result of the particle size distribution of Staphylococcus (2) (before after-culture -).
[Drawing 33]The analysis result of the particle size distribution of streptococci (1) (before culture).
[Drawing 34]The analysis result of the particle size distribution of streptococci (1) (after culture).
[Drawing 35]The analysis result of the particle size distribution of streptococci (1) (before after-culture -).
[Drawing 36]The analysis result of the particle size distribution of streptococci (2) (before culture).
[Drawing 37]The analysis result of the particle size distribution of streptococci (2) (after culture).
[Drawing 38]The analysis result of the particle size distribution of streptococci (2) (before after-culture -).
[Drawing 39]The analysis result of the particle size distribution of streptobacillus (before culture).
[Drawing 40]The analysis result of the particle size distribution of streptobacillus (after culture).
[Drawing 41]The analysis result of the particle size distribution of streptobacillus (before after-culture -).
[Drawing 42]The analysis result of the particle size distribution of yeast-like fungi (before culture).
[Drawing 43]The analysis result of the particle size distribution of yeast-like fungi (after culture).
[Drawing 44]The analysis result of the particle size distribution of yeast-like fungi (before after-culture -).
[Drawing 45]The mimetic diagram showing the difference in the particle size distribution by the strain of Staphylococcus, streptococci, streptobacillus, and a Bacillus.

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CLAIMS

[Claim(s)]
[Claim 1]A measuring method of a microorganism in a culture sample which added a sample of a measuring object to culture medium characterized by comprising the following.
The 1st measuring process that measures said culture sample after carrying out predetermined time culture processing using said flow cytometer, and acquires the 1st particle size distribution of said culture sample from optical information of the scattered light detected.
The 2nd measuring process that measures a culture sample before said culture processing using said flow cytometer, and acquires the 2nd particle size distribution of said culture sample from optical information of the scattered light detected.
An analysis process which acquires particle size distribution of a microorganism cultivated in said culture sample from difference of said 1st particle size distribution and the 2nd particle size distribution.
An output process which outputs particle size distribution of said obtained microorganism.

[Claim 2]The microorganism measuring method according to claim 1 which measures emission time and intensity of a scattered-light signal which are detected using said flow cytometer, and is characterized by acquiring said 1st and 2nd particle size distribution.
[Claim 3]Particle size distribution of said cultivated microorganism is acquired by measuring emission time and intensity of a scattered-light signal which are detected using said flow cytometer, The microorganism measuring method according to claim 1 which presumes vegetative form voice of said microorganism based on said particle size distribution, and is characterized by judging to any of a predetermined classification said microorganism belongs.
[Claim 4]Particle size distribution of said cultivated microorganism is acquired by measuring emission time and intensity of a scattered-light signal which are detected using said flow cytometer, The microorganism measuring method according to claim 1 judging to any vegetative form voice of said microorganism shall belong between Staphylococcus, streptococci, streptobacillus, or a Bacillus based on said particle size distribution.
[Claim 5]The microorganism measuring method according to claim 1, wherein a measuring object sample is urine.
[Claim 6]The microorganism measuring method according to claim 1 characterized by measuring bacteria and/or yeast-like fungi as a microorganism.
[Claim 7]A microorganism measuring device comprising:
The 1st measuring means that acquires the 1st particle size distribution of a culture sample after performing predetermined time culture processing from optical information of the scattered light which is used with flow cytometer which measures a particle size of a microorganism in a culture sample which added a measuring object sample to culture medium, and is detected by said flow cytometer.

The 2nd measuring means that acquires the 2nd particle size distribution of a culture sample before said culture processing from optical information of the scattered light detected by said flow cytometer.
An analysis means to acquire particle size distribution of a microorganism cultivated in said culture sample from difference of said 1st particle size distribution and the 2nd particle size distribution.
An output means which outputs particle size distribution of said obtained microorganism.

[Claim 8]It is used with flow cytometer which measures a particle size of a microorganism in a culture sample which added a measuring object sample to culture medium, It is the recording medium which recorded an analysis program which analyzes a measurement result of said particle size and in which computer reading is possible, A; A stage of acquiring the 1st particle size distribution of a culture sample after performing predetermined time culture processing from optical information of the scattered light detected by said flow cytometer, B; A stage of acquiring the 2nd particle size distribution of a culture sample before said culture processing from optical information of the scattered light detected by said flow cytometer, A stage of acquiring particle size distribution of a microorganism cultivated in said culture sample based on difference of the 1st particle size distribution of C; above, and the 2nd particle size distribution, and D; a recording medium which recorded an analysis program for performing a stage which outputs particle size distribution of said obtained microorganism and in which computer reading is possible.

[Translation done.]